

# Improvement of activity-related knee joint discomfort following supplementation of specific collagen peptides

Denise Zdzieblik, Steffen Oesser, Albert Gollhofer, and Daniel König

**Updated online 18 April 2017:** The license for this article has been changed to the CC BY 4.0 license. The PDF and HTML versions of the article have been modified accordingly.

**Abstract:** The aim of the study was to evaluate the use of specific collagen peptides in reducing pain in athletes with functional knee problems during sport. Athletic subjects ( $n = 139$ ) with functional knee pain ingested 5 g of bioactive collagen peptides (BCP) or a placebo per day for 12 weeks. The primary outcome of the study was a change in pain intensity during activity, which was evaluated by the participants and the attending physicians using a visual analogue scale (VAS). As secondary endpoints, pain intensity under resting conditions, the range of motion of the knee joint, and the use of additional therapeutic options were assessed. The results revealed a statistically significant improvement in activity-related pain intensity in the verum group compared with placebo. ( $\Delta\text{VAS}_{\text{BCP}} = 19.5 \pm 2.4$ ;  $\Delta\text{VAS}_{\text{placebo}} = 13.9 \pm 2.1$ ;  $p = 0.046$ ). The results were confirmed by the physician's assessment. ( $\Delta\text{VAS}_{\text{BCP}} = 16.7 \pm 1.8$ ;  $\Delta\text{VAS}_{\text{placebo}} = 12.2 \pm 1.8$ ;  $p = 0.021$ ). Pain under resting conditions was also improved, but no significance compared with placebo was detected ( $\Delta\text{VAS}_{\text{BCP}} = 10.2 \pm 18.4$ ;  $\Delta\text{VAS}_{\text{placebo}} = 7.4 \pm 15.2$ ;  $p = 0.209$ ). Due to the high joint mobility at baseline, no significant changes of this parameter could be detected. The use of additional treatment options was significantly reduced after BCP intake. The study demonstrated that the supplementation of specific collagen peptides in young adults with functional knee problems led to a statistically significant improvement of activity-related joint pain.

**Key words:** collagen peptides, collagen hydrolysate, athletes, functional joint discomfort, visual analogue scale.

**Résumé :** Cette étude a pour objectif d'évaluer l'utilisation de peptides de collagène spécifiques pour la diminution de la douleur chez des athlètes présentant des anomalies fonctionnelles du genou dans le sport. Cent-trente-neuf athlètes présentant une douleur fonctionnelle au genou consomment tous les jours 5 g de peptides bioactifs de collagène (BCP) ou un placebo durant 12 semaines. Le résultat principal de cette étude est la modification, durant l'activité, de l'intensité de la douleur telle qu'évaluée par les participants et le médecin sur place au moyen d'une échelle visuelle analogue (VAS). On évalue aussi, dans un deuxième temps, l'intensité de la douleur au repos, l'amplitude de mouvement du genou et l'utilisation de traitements additionnels. Les résultats révèlent une diminution statistiquement significative de l'intensité de la douleur à l'effort dans le groupe expérimental comparativement au groupe de contrôle ( $\Delta\text{VAS}_{\text{BCP}} = 19,5 \pm 2,4$ ;  $\Delta\text{VAS}_{\text{placebo}} = 13,9 \pm 2,1$ ;  $p = 0,046$ ). Les résultats confirment l'évaluation du médecin ( $\Delta\text{VAS}_{\text{BCP}} = 16,7 \pm 1,8$ ;  $\Delta\text{VAS}_{\text{placebo}} = 12,2 \pm 1,8$ ;  $p = 0,021$ ). La douleur au repos est aussi diminuée, mais pas de façon significative par rapport au groupe de contrôle ( $\Delta\text{VAS}_{\text{BCP}} = 10,2 \pm 18,4$ ;  $\Delta\text{VAS}_{\text{placebo}} = 7,4 \pm 15,2$ ;  $p = 0,209$ ). On n'enregistre pas de modifications significatives de la mobilité du genou probablement à cause de la mobilité élevée de l'articulation au début de l'expérimentation. À la suite de la consommation de BCP, on note une diminution significative des traitements additionnels. D'après cette étude, la supplémentation en peptides de collagène spécifiques chez de jeunes adultes présentant des anomalies fonctionnelles du genou suscite une diminution statistiquement significative de la douleur articulaire associée à l'exercice physique. [Traduit par la Rédaction]

**Mots-clés :** peptides de collagène, hydrolysats de collagène, athlètes, malaises articulaires fonctionnels, échelle visuelle analogue.

## Introduction

Collagen peptides are derived from an enzymatic hydrolysis of collagen, consisting mainly of the amino acids glycine (Gly), proline (Pro), and hydroxyproline (Hyp) (Clemente 2000; Dybka and Walczak 2009; Oesser and Seifert 2003; Walrand et al. 2008; Watanabe-Kamiyama et al. 2010). The amino acids sequence and the molecular weight distribution of the peptides depends on the raw materials source and the specific production process (Iwai et al. 2005; Ohara et al. 2007; Saito et al. 2001). Collagen peptides are classified as a safe food by the European Food Safety Authority

(European Food Safety Authority 2005) and by the Food and Drug Administration (FDA (U.S. Food and Drug Administration) 2003). It has been found that collagen peptides are almost completely reabsorbed in the small intestine (Iwai et al. 2005; Ohara et al. 2007; Walrand et al. 2008; Watanabe-Kamiyama et al. 2010). About 10% of the collagen fragments are taken up in peptide form with a size of 1 to 10 kDa. These peptides are directly transferred from the gastrointestinal tract into the blood (Iwai et al. 2005; Ohara et al. 2007; Walrand et al. 2008; Watanabe-Kamiyama et al. 2010). According to the results of preclinical trials, a significant amount of these peptides accumulate in the articular cartilage (Oesser et al. 1999).

Received 14 July 2016. Accepted 10 January 2017.

**D. Zdzieblik.** Department of Nutrition, University of Freiburg, Department of Sport and Sport Science, Schwarzwaldstraße 175, 79117 Freiburg, Germany.

**S. Oesser.** CRI, Collagen Research Institute, Kiel, Germany, Schauenburgerstraße 116, 24118 Kiel.

**A. Gollhofer and D. König.** University of Freiburg, Department Sport and Sport Science, Schwarzwaldstraße 175, 79117 Freiburg, Germany.

**Corresponding author:** Denise Zdzieblik (email: [denise.zdzieblik@sport.uni-freiburg.de](mailto:denise.zdzieblik@sport.uni-freiburg.de)).

Copyright remains with the author(s) or their institution(s). This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

On the basis of recent studies, a joint-protecting effect of collagen peptides is discussed. In the study by Oesser and Seifert, a positive effect of collagen peptides on the regeneration of hyaline cartilage was observed in cultured chondrocytes (Oesser and Seifert 2003). The experiments showed a direct, dose-dependent stimulation of type II collagen and proteoglycans by the addition of collagen peptides. The stimulation of the extracellular matrix (ECM) synthesis is probably caused by specific Hyp-Pro-Gly containing peptides with a molecular size <10 kDa (Ng et al. 2007; Oesser and Seifert 2003; Walrand et al. 2008; Watanabe-Kamiyama et al. 2010). Other in vitro experiments showed an increase of protease activity after collagen fragments supplementation (Fichter et al. 2006; Jennings et al. 2001). There is evidence that the effects of collagen hydrolysate depend on the concentration, the experimental conditions, (Ng et al. 2007), and the characteristic of the tested collagen hydrolysates (Schadow et al. 2013). However, the clinical relevance of the observed effects needs to be clarified. In a placebo-controlled trial, McAlindon et al. (2011) demonstrated increased content of proteoglycan as part of the ECM of cartilage tissue in patients with mild osteoarthritis after 10 g of collagen peptide intake. In addition, clinical studies with patients suffering from degenerative joint diseases showed an improvement in pain symptoms (Bello and Oesser 2006; Benito-Ruiz et al. 2009; Bruyère et al. 2012; Moskowitz 2000). It has to be assumed that functional joint discomforts are characterized by a subclinical short-term degradation of cartilage due to the increased stress on the knee joint. Athletes aged 15–30 especially suffer from stress-induced knee joint complaints (Boling et al. 2010; Witvrouw et al. 2014).

In a placebo-controlled trial by Clark et al. (2008), the efficacy of the daily intake of collagen peptides was tested in young athletes suffering from joint discomfort. The results suggested a reduction in joint pain during physical activity, especially in the knee joint. However, there were no significant differences between the 2 study groups after adjustment for multiple testing. Therefore, more clinical trials are needed to elucidate the role of collagen peptide supplementation as a possible treatment option in that particular target group.

In the present study, the change in activity-related knee pain intensity was investigated in young sporting individuals. In a randomized controlled design, specific collagen peptides or a placebo were supplemented over a study period of 12 weeks.

## Methods

### Study design

The study was a monocentric, prospective, randomized, double-blind, placebo-controlled, phase III trial carried out at the University of Freiburg. The effect of a daily dosage of 5 g of collagen peptides (FORTIGEL, GELITA AG, Germany) on joint pain and flexibility was investigated in individuals with activity-related knee joint discomfort. They are defined as nonstructural knee complaints that do not occur as a result of injury or primary joint disorders (e.g., inflammation, osteoarthritis) but develop as a consequence of overloading or incorrect loading of the knee during physical activity. In total, 160 women and men aged 18–30 and engaged in sporting activities were recruited. The sample size was determined by a power calculation based on the data (bioactive collagen peptides (BCP):  $n = 29$ ,  $\Delta$ VAS (visual analogue scale) =  $-1.38 \pm 2.12$  vs. placebo:  $n = 34$ ,  $\Delta$ VAS =  $-0.54 \pm 1.65$ ) of a previous trial on athletes with functional knee issues (Clark et al. 2008). For an intended test power of 80% with a significance level  $\alpha = 0.05$  and an estimated dropout rate of 10%, 80 individuals were included per study group. Participants were athletes or sports students of the University of Freiburg, who had to exercise regularly for at least 3 hours per week. The knee joint discomfort should not result from any injury or degenerative or inflammatory joint disease. The intervention period was 12 weeks. Overall, 3 visits took place; at the beginning, after 6 weeks, and at the end of the inter-

vention period ( $t_0$ ,  $t_6$ ,  $t_{12}$ ). The study was conducted with the approval of the Ethics Committee of the Medical Faculty of the University of Freiburg. All participants gave written informed consent. For enrollment, inclusion and exclusion criteria were checked by an experienced physician using anamnestic data, clinical examinations, and blood testing. In addition, participants were asked to bring radiographs or the reports of previous medical examinations.

If there was no indication for structural issues by a former clinical diagnosis or the physician's assessment during the initial visit, subjects were included. Potential participants were excluded from the trial if at least one of the following criteria was present:

- primary knee joint disease (osteoarthritis, rheumatic or bacterial/viral inflammation),
- known hypersensitivity to the supplements,
- intra-articular injections (cortisol, hyaluronic acid, etc.) during the last 6 months,
- ingestion of supplements such as glucosamine or chondroitin during the last 6 months,
- intake of hydrolysed collagen in the last 6 months,
- extreme pain symptoms requiring a high-dose analgesic therapy over a longer period of time (>2 weeks) or an intra-articular injection treatment.

Subjects were assigned to the BCP or placebo group by the investigator using a web-based random number generator (Research Randomizer, n.d.). Maltodextrin was used as a placebo. Both investigational products (similar in taste and identical in design) were provided in a blind manner coded by a number. The coding was not broken until all data were entered, the dataset was secured, and the statistical analysis was performed. Subjects were instructed not to change their usual lifestyle and, particularly, to maintain their dietary habits and habitual level of physical activity. Furthermore, participants were asked not to add further treatment options after the initial visit.

The supplementation of BCP or placebo was documented by using a compliance calendar.

### Efficacy outcomes

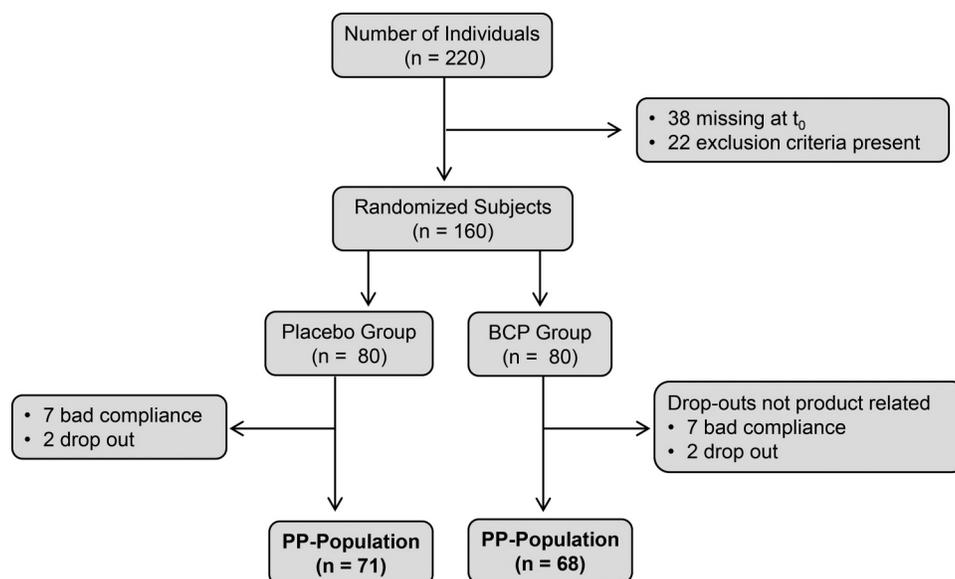
The primary endpoints of the study were defined as changes in pain intensity during activity after 12 weeks of supplementation assessed by the study participants and the attending physicians. A VAS was used for measurements at all visits. At the baseline visit, the VAS-score of activity-related pain assessed by the subject had to be at least 20 mm on a scale ranging from 0 to 100 mm.

As a secondary outcome, the changes in pain at rest evaluated by subjects, alterations in knee joint mobility, as well as the use of additional treatments (classified as drugs, bandages, physiotherapy, combined therapies, and others) were recorded. Joint mobility (flexion and extension) was evaluated by using the range of motion (ROM) method. Starting from the zero position (basic position of the knee joint in upright position), the maximum extension (first digit) and the maximum flexion (third digit) were recorded (Aumüller et al. 2010). In addition, the affected knee was examined at every visit to exclude structural knee damage. The following tests were carried out:

- McMurray test (McMurray 1942) (in conjunction with Bragard) for a meniscus injury,
- Steinman test (F. Steinmann, 1929) (I & II) to ascertain instability of the medial and lateral collateral ligament,
- the drawer test (Butler et al. 1980) for the anterior and posterior cruciate ligament.

As a safety parameter and to exclude any inflammatory processes, blood parameters were analyzed at the beginning ( $t_0$ ) and the end ( $t_{12}$ ) of this study.

**Fig. 1.** Flow chart of subject recruitment and drop-outs before and during the study. BCP, bioactive collagen peptides;  $t_0$ , start of intervention; PP, per protocol.



### Statistical analysis

Data are represented as mean  $\pm$  SEM. All statistical analyses were done using SPSS statistics 20 and the significance level was set at  $\alpha = 0.05$  (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

The baseline values of all parameters were compared between the study groups to show accordance for demographic and anamnestic parameters. Testing for ordinal baseline values was performed with the Mann-Whitney U-test. Dichotomous baseline values were tested by the  $\chi^2$  test.

The Wilcoxon test for non-interval-scaled paired samples was used to test whether the changes in pain intensity or range of motion during the course of the study were significantly different within study groups at the points of examination ( $t_{0-6}$ ,  $t_{6-12}$ , and  $t_{0-12}$ ). Differences between the BCP and the placebo group were analyzed with the Mann-Whitney U-test for non-interval scale non-affiliated paired data.

All tests within the descriptive analysis were performed on a 2-sided basis, and the level of significance was assessed to  $\alpha = 0.05$  at any one time. The results were exclusively hypothesis-generating and did not have any confirmatory character.

The number of alternative treatment options was listed in a frequency table and compared between the 2 study groups. The  $\chi^2$  test by McNemar for dichotomous paired samples was used to analyze the significance of terminating alternative therapies in each study group. As no hierarchy for the 2 primary endpoints had been defined in the study protocol, an analysis according to Bonferroni-Holm was performed. The  $p$  values were listed in ranking order. The smallest  $p$  value was compared against  $\alpha/2$  ( $= 0.025$ ) and the second smallest against  $\alpha/1$  ( $= 0.05$ ).

### Results

In all, 220 people were screened for this clinical intervention (Fig. 1). Of this population, 160 men and women met the inclusion criteria of the study and were randomized. A total of 139 subjects completed the study. The reasons for premature study termination are presented in Fig. 1. None of the drop-outs was related to any side effects caused by the intake of the collagen peptide supplement or placebo. No adverse events were noted, and in particular, no pathological findings were observed in routine blood testing at the beginning and the end of this study, which indicated any adverse side effects due to the intake of the collagen peptides or of the placebo.

**Table 1.** Baseline characteristics of the study population.

	BCP (n = 68)	Placebo (n = 71)	<i>p</i>
Age (y)	24.1 $\pm$ 0.3	24 $\pm$ 0.3	0.762
Gender (male/female)	25/43	31/40	0.407*
Height (m)	1.73 $\pm$ 0.1	1.74 $\pm$ 0.1	0.267
Weight (kg)	67.6 $\pm$ 1.3	68.2 $\pm$ 1.5	0.896
BMI (kg/m <sup>2</sup> )	22.6 $\pm$ 0.3	22.3 $\pm$ 0.3	0.324
BP sys (mm Hg)	123.5 $\pm$ 1.5	125.4 $\pm$ 1.0	0.281
BP dia (mm Hg)	75.2 $\pm$ 1.5	75.5 $\pm$ 1.5	0.673

**Note:** Data are presented as mean  $\pm$  SE. BMI = body mass index, BP sys, dia = blood pressure systolic, diastolic.

\* $\chi^2$  test not significant.

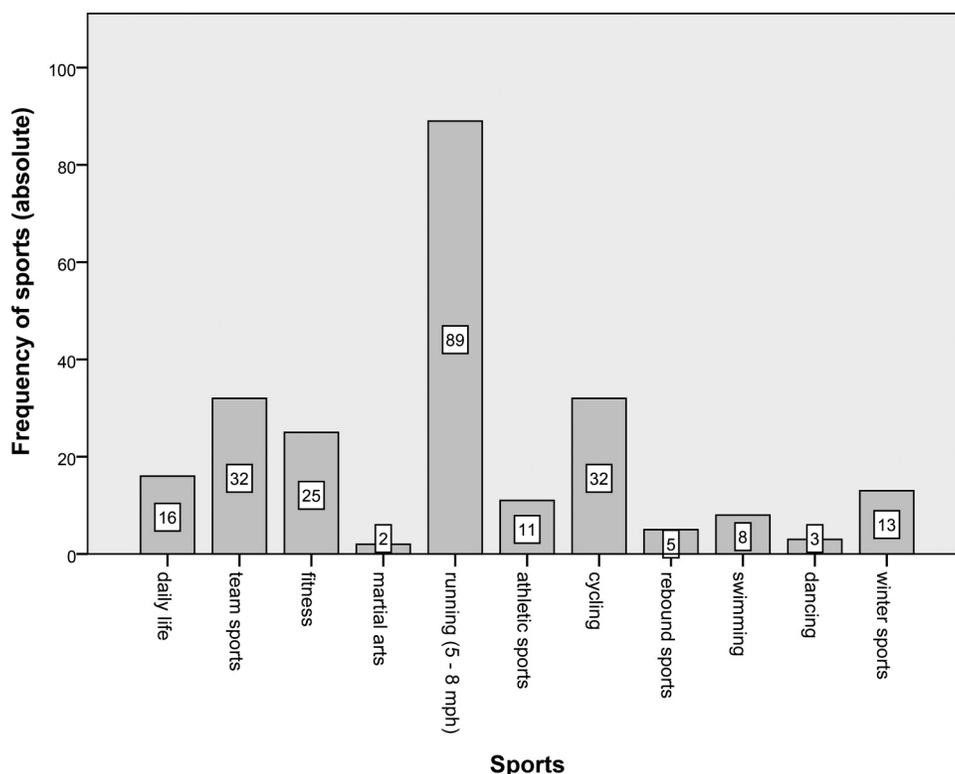
### Baseline characteristics

To evaluate whether both study groups were homogeneous regarding the baseline data, the Mann-Whitney U-test (non-interval scale samples) and the  $\chi^2$ -test were performed (Table 1). No statistically significant baseline differences between the BCP and placebo group were detected. Although more women ( $n = 83$ ) than men ( $n = 56$ ) were present in the study population, the gender distribution was not statistically significantly different between the placebo and the treatment group.

### Changes in knee pain

The anamnesis of the initial physical examination indicated that 47% of the participants in the evaluated study population had knee joint pain directly during activity. In 22% of the subjects, the pain occurred immediately after activity and remained for at least 90 min. Thirty-two percent of the athletes had knee joint pain during and after physical activity. For estimation, the maximum value of pain was taken. In the majority of the test persons (58%), both knees were affected. As part of the medical history, the subjects were asked which sports triggered the knee joint discomforts (Fig. 2). The participant survey showed that most stress-induced knee pain occurred in endurance, rebound, and team sports. Furthermore the origins of knee pain were examined. In this study, an inadequate or overloading stress of the knee joint led to functional discomforts in 79 cases (50%). Some of the study participants (10.8%) ( $n = 15$ ) named anatomical deformities (leg length differences or misaligned joints) as cause of their discomforts.

**Fig. 2.** Absolute number of pain-causing activities. Data show the absolute case number. Some participants performed several activities causing knee joint discomforts. In addition, everyday situations such as climbing stairs were included in the evaluation. MPH, miles per hour.



### Sports

Changes in knee pain were evaluated by VAS scores as assessed by the participants and the attending physicians.

At the baseline level, no statistically significant differences between the test groups could be detected ( $p = 0.766$ ). All individuals reported a pronounced activity-related pain ( $50.1 \pm 17.0$  in the BCP group;  $49.7 \pm 17.5$  for placebo). This assessment was confirmed by attending physicians. Here an initial VAS score of  $46.2 \pm 9.5$  was evaluated for the treatment group and  $48.0 \pm 10.4$  for the placebo group (group differences  $p = 0.225$ ).

During the course of the study, knee pain was statistically significantly ( $p < 0.001$ ) reduced in the collagen peptide treated group and in the placebo group after 6 weeks, as assessed by the participants and the attending physician (Figs. 3 and 4). This reduction in pain continued in both groups until the end of the study after 12 weeks. However, the effect seems to be more pronounced in the BCP group (38.4%) compared with placebo (27.9%) (Table 2).

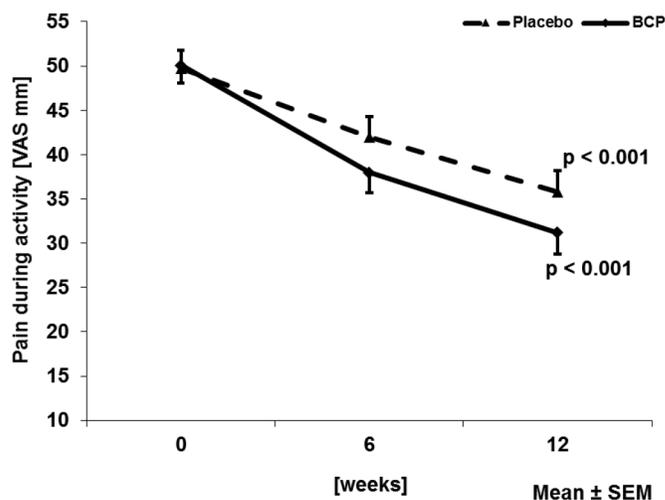
To verify the observed differences between the treatment and the placebo group, a statistical analysis using the Mann-Whitney U-test was carried out comparing the changes in the VAS scores from the beginning ( $t_0$ ) to the end of the study ( $t_{12}$ ).

It could be demonstrated that treatment with collagen peptides led to a statistically significant reduction in the VAS scores compared with placebo for both primary study endpoints “pain during activity”, as assessed by the participants ( $p = 0.046$ ), and “pain during activity”, assessed by attending physicians ( $p = 0.021$ ) (Fig. 5).

For the secondary endpoint “pain at rest”, participants reported much lower mean pain score values compared with the activity-related pain. In the treatment group, a mean VAS score of only  $20.8 \pm 22.4$  was measured. Compared with placebo ( $17.1 \pm 22.5$ ), no statistically significant group differences could be detected ( $p = 0.253$ ) at baseline.

Thirty-seven percent of the participants in the treatment group and 44% of the individuals in the placebo group had no rest pain at the beginning of the study.

**Fig. 3.** Changes in pain during activity assessed by the study participants. Data are presented as mean  $\pm$  SEM for  $n = 68$  (BCP group) and  $n = 71$  (placebo group). BCP, bioactive collagen peptides.

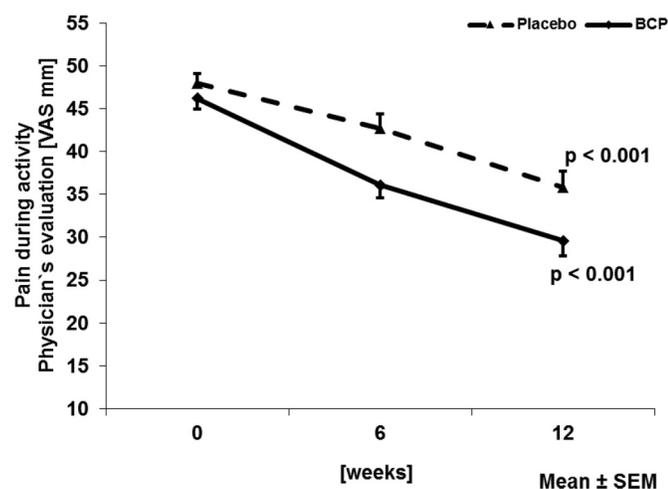


Nevertheless, in total a statistically significant ( $p < 0.01$ ) improvement of “pain at rest” was observed in the collagen peptide group and in the placebo group during the course of the study. Although the pain reduction detected was more pronounced in the treatment group ( $10.2 \pm 18.4$ ) compared with placebo ( $7.4 \pm 15.2$ ), the difference was not statistically significant ( $p = 0.209$ ).

As an additional secondary outcome of the study, the improvement of the knee joint mobility was investigated using the ROM method.

The data revealed no statistically significant differences for knee joint extension and flexion between the study groups at

Fig. 4. Changes in pain during activity assessed by the attending physicians. Data are presented as mean  $\pm$  SEM for  $n = 68$  (BCP group) and  $n = 71$  (placebo group). BCP, bioactive collagen peptides.



baseline. Since none of the study participants had restricted mobility of the knee joint at the beginning of the study, no changes in knee extension and flexion could be observed in either study group during the 12 week treatment, as expected. The data revealed no statistically significant differences between the collagen peptide treatment and the placebo at the end of the trial (data not shown).

#### Use of additional therapies

In total, 47 subjects (34%) had used alternative therapies, which were divided into 5 groups: drugs (Ibuprofen 400; Voltaren), bandages, physiotherapy, combined therapies, and others (Table 3). At the beginning of the trial, 27 subjects in the intervention and 20 subjects in the placebo group had used additional therapies. At baseline, the 2 study groups did not significantly differ statistically ( $p = 0.151$ ). After the 12 week duration of the study, the need for additional therapies was statistically significantly reduced in both study groups. The data suggested that the reduction in the BCP group was more pronounced (59%) compared to the results in the placebo group (40%) (Table 3).

#### Discussion

In several clinical trials, the efficacy of oral collagen peptide supplementation could be demonstrated in osteoarthritic patients (McAlindon et al. 2011; Benito-Ruiz et al. 2009; Adam 1991; Krug 1979; Oberschelp 1985; Bruyère et al. 2012). After treatment, significantly reduced pain and increased mobility were reported. Much less data exists on the impact of a collagen peptide therapy in individuals suffering from activity-related joint discomfort due to excessive physical activity and not caused by osteoarthritis (OA). To date, the most valid information comes from an RCT conducted by Clark et al. (2008), investigating the effect of a collagen peptide intake on joint discomfort in 147 varsity athletes at Penn State University. The results revealed a positive effect of the collagen peptide intake on several joints. Although the effect sizes indicated a clear improvement, the data failed to reach the level of statistical significance compared to placebo due to the challenging study design with multiple primary endpoints.

Therefore, the current clinical trial on physically active young adults with activity-related joint pain focused exclusively on the effect of a daily intake of collagen peptides on the knee joint. The primary endpoints of the study revealed a pronounced, statistically significant ( $p < 0.05$ ) reduction in "pain during activity" after collagen peptide treatment compared to placebo, as assessed by the study participants and the attending physicians. The results

confirm in principle findings from previous investigations into young athletes using the identical collagen peptide product (Clark et al. 2008; Flechsenhar and Alf 2005). The calculated effect size ( $d = 0.300$ ) of a BCP treatment compared with placebo was similar to the results of the Clark study, although in the current investigation the daily dosage was reduced from 10 to 5 g, and the duration of the treatment was shortened from 24 to 12 weeks. The dose reduction to 5 g was based on the observation that in clinical trials in OA patients (Adam 1991; Benito-Ruiz et al. 2009; Bruyère et al. 2012; Carpenter et al. 2005; Krug 1979; McAlindon et al. 2011; Moskowitz 2000; Oberschelp 1985), and the efficacy of the collagen peptide intake seems to be independent from BMI of the subjects. This indicates that a daily intake of 10 g might not be required in subjects with OA. As the participants in this study were suffering from activity-related joint discomfort with no signs any degenerative joint disease, the dosage was reduced by 50%.

The efficacy of a 12 week BCP treatment could be demonstrated, and interestingly, the physicians and the participants' assessments of the activity-related knee pain were very similar. A pain reduction of 38% was measured after the collagen peptide treatment according to the participants' evaluation ( $p = 0.046$ ;  $d = 0.298$ ; compared to placebo). The assessment of the attending physicians revealed a pain reduction of 36% ( $p = 0.021$ ;  $d = 0.302$ ; compared to placebo) after the BCP supplementation, with a remarkably high responder rate of 88%.

In the current study, the impact on rest pain was also investigated as a secondary outcome. Although a pronounced pain reduction of 49% was observed after BCP treatment, the difference was not statistically significant compared with placebo. This might be explained by the fact that the reported level of rest pain was remarkably low at the beginning of the study, with VAS scores of  $20.8 \pm 22.4$  in the treatment group and  $17.1 \pm 22.5$  in the placebo group. Forty percent of the participants had no pain whatsoever at rest. These limited opportunities for pain improvement and high standard deviations of the analyzed data might explain the lack of statistical significance, although the effect size ( $d = 0.339$ ) was comparable to the pain improvement during activity. This finding confirms earlier results from a study on sport students at Penn State University (Clark et al. 2008). Here the study participants also reported only slight rest pain. Pronounced activity-related pain levels, together with minor "pain at rest", seem to be typical for younger athletes who expose their knee joints to a certain stress due to excessive sports. In contrast, it is known that among elderly people suffering from degenerative joint diseases, rest pain along with limited joint mobility are the predominate problems (Piedras-Jorge et al. 2010).

Apart from the changes in VAS scores, the differences of joint mobility were examined using the ROM method. As expected, the participating athletes' joint mobility was hardly affected at baseline, which explains why no significant improvement in knee joint flexion and extension could be observed during the course of the study. Mobility was not restricted, because there is no severe and progressive degradation of cartilage of the knee joint. Accordingly, there were sufficient intact collagen and proteoglycan networks for stabilization and for the flexibility of the articular cartilage available. Therefore, it is assumed that exercise-induced knee pain and a limitation of joint mobility may not necessarily occur together.

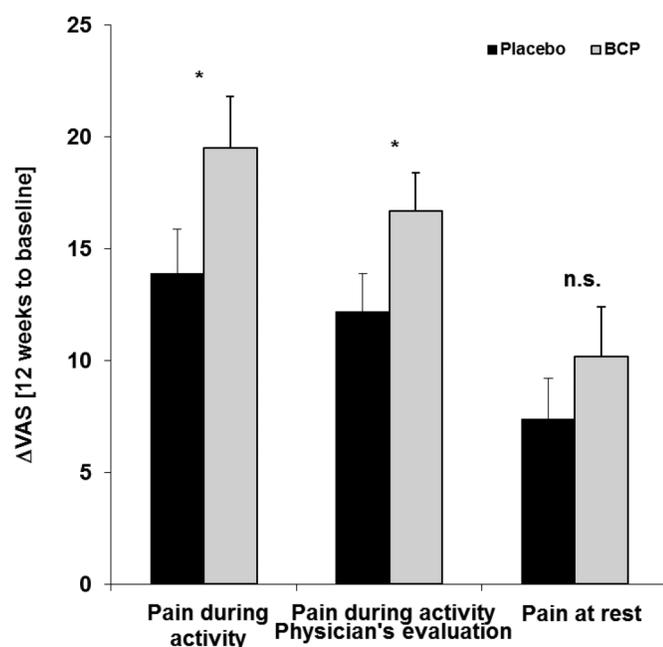
However, the survey of the participants has shown a significantly less use in therapies like physiotherapy or ice packs after the treatment with BCP. The discontinuation of medication for pain relief can be attributed to the significant decrease in pain intensity. Although this positive trend was also observed in the placebo group, the effect was more pronounced with a tendency towards significance ( $p = 0.07$ ) in the BCP group after the collagen peptide intake. In the treatment group, 60% of the subjects that had used additional therapies were able to stop these treatments

**Table 2.** Changes in knee joint pain after 12 weeks.

	Group	n	$\bar{X}_0 \pm \text{SEM}_0$	$\bar{X}_{12} \pm \text{SEM}_{12}$	VAS	Value	$d_{\text{Cohen}}$
<b>Primary endpoint</b>							
Pain during activity	BCP	68	50.1±2.1	31.2±2.4*	19.5±2.4	0.046**	-0.298
	Placebo	71	49.7±2.1	35.8±2.5*	13.9±2.1		
Pain during activity (physician's evaluation)	BCP	68	46.2±1.2	29.6±1.9*	16.7±1.8	0.021**	-0.302
	Placebo	71	48.0±1.2	35.8±1.8*	12.2±1.8		
<b>Secondary endpoint</b>							
Pain at rest	BCP	68	32.9±3.0	15.7±3.0*	10.2±2.2	0.209	-0.339
	Placebo	71	30.4±3.5	17.3±3.3*	7.4±1.8		

**Note:** Data are presented as mean VAS Scores  $\pm$  SEM at baseline ( $t_0$ ) and after 12 weeks ( $t_{12}$ ). \* $p < 0.001$  for an intra-group analysis. \*\*Significant group differences after Bonferroni–Holm correction.  $d_{\text{Cohen}}$  = effect size compared with placebo. VAS, visual analogue scale; BCP, bioactive collagen peptides.

**Fig. 5.** Differences of VAS scores of the primary outcomes, “pain during activity” evaluated by the participants and by the attending physicians as well as for the secondary outcome “pain at rest” after a 12-week treatment. Data are presented as mean  $\pm$  SEM for  $n = 68$  (BCP group) and  $n = 71$  (placebo group). \*Significant group differences after Bonferroni–Holm correction. n.s., not statistically significant; VAS, visual analogue scale; BCP, bioactive collagen peptides.

**Table 3.** Additional therapy options used.

	BCP (n = 68)		Placebo (n = 71)	
	$t_0$	$t_{12}$	$t_0$	$t_{12}$
Drugs	3	0	4	0
Bandages	7	4	7	3
Physiotherapy	3	0	3	3
Others	12	6	6	6
Combined therapies	2	1	0	0
Sum	27	11	20	12
$p$	<0.001		0.021	

**Note:** Data are presented as absolute numbers at baseline ( $t_0$ ) and after 12 weeks ( $t_{12}$ ). BCP, bioactive collagen peptides.

during the course of the study. In the placebo group, only 40% had no further need for additional therapies after 3 months.

The use of collagen peptides in the treatment of joint problems is still the subject of scientific discourse. Among other things, the

recommended dosage and the duration of treatment are discussed. In most of the clinical trials on OA patients, a daily dosage of 10 g collagen peptides over 3 months was effective to significantly reduce pain and improve mobility compared with placebo (Bello and Oesser 2006; Benito-Ruiz et al. 2009; Bruyère et al. 2012; Aumüller et al. 2010).

The exact mechanism of the clinical efficacy of collagen peptide supplementation is still the subject of research. In preclinical experiments, it could be demonstrated that specific bioactive collagen peptides stimulate type II collagen and proteoglycan synthesis in articular cartilage (Ng et al. 2007; Oesser and Seifert 2003). This stimulatory effect could counteract wear and tear processes of the cartilage tissue and might help to repair micro injuries. Consequently, this decreased ECM degradation could reduce pro-inflammatory and pain-stimulating processes. A direct anti-inflammatory potential of collagen peptides is discussed that could also lead to decreased joint pain intensity. This hypothesis is supported by preclinical experiments, which have demonstrated that collagen peptide supplementation induces a glycine-mediated inhibition of cytokine release (Hartog et al. 2013).

Other in vitro experiments confirm the impact of collagen fragments on cartilage metabolism, indicating an increase of protease activity and thus a regulation of matrix turnover by controlling a catabolic pathway (Fichter et al. 2006; Jennings et al. 2001). The specific effects seems to be dependent on the collagen hydrolysate concentration, the experimental conditions (Ng et al. 2007), and specific the characteristic of the tested collagen hydrolysates (Schadow et al. 2013).

The best clinical data with regard to the possible mode of action come from McAlindon et al. (2011). In this study a direct positive effect of specific collagen peptides on the knee cartilage could be demonstrated. The data revealed a statistically significant increase in the proteoglycan content compared with placebo. In a very recent re-analysis of the blood samples of the same study participants, a statistically significant increase in type II collagen synthesis together with a significantly reduced proteoglycan degradation could be detected (McAlindon et al. 2017). The results indicate an impact on cartilage metabolism that might help to counteract progressive wear and tear processes.

However, the extent of cartilage loss varies greatly, depending on the severity of osteoarthritis. In functional knee problems, it could be speculated that only a subclinical physiological short-term degradation of cartilage needs to be repaired. Therefore, the reduction of functional joint pain probably needs a shorter period of intervention and a lower dose of collagen peptides.

Besides, the stimulatory impact seems to be dependent on the specification of the collagen peptides administered. It could be demonstrated that different collagen hydrolysates differ in their physico-chemical properties, which could have an impact on the interaction of the peptides with certain integrin receptors (Siebert et al. 2010; Stötzel et al. 2012). Molecular weight distribution of the collagen peptides and the specific amino acid sequences might be of importance for the efficacy play a major role, whereas the

collagen source seems to be of minor importance (Kumar et al. 2015). As a consequence, the currently demonstrated clinical efficacy based on a daily intake of a 5 g BCP is only valid for this specific collagen peptide product, and results should not be extrapolated to the use of collagen peptides per se.

Future studies might evaluate the optimal dosage of collagen peptide for the treatment of activity-related joint discomfort in athletes. The efficacy of these peptides in an elderly, non-athletic population with functional joint discomfort would also be of particular interest.

## Conclusion

The current study on young, physically active individuals demonstrated that the daily intake of 5 g of collagen peptides led to a statistically significant reduction in activity-related knee joint pain after a 12 week treatment compared with placebo. The improvement on joint discomfort was also accompanied by a statistically significantly reduced need for additional therapies such as physiotherapy or ice packs.

Overall, the study confirmed the efficacy of collagen peptide intake on activity-related knee joint discomfort making it a potentially interesting option for the treatment of joint pain induced by physical stress.

## Acknowledgement

We would like to thank all the participants as well as the staff of the Institute of Preventive Medicine who helped with collecting the data, and Dr. Markus Wenning, who supported us in the clinical examination. DZ, DK, SO, as well as AG conceived and designed the study. DZ and DK were responsible for data acquisition. Analysis was undertaken by DZ and DK. All authors interpreted data, drafted or revised the article critically for important intellectual content, and approved the final version of the manuscript. This research was funded by GELITA Health GmbH, Eberbach Germany. The authors have neither financial nor competing interests concerning the outcome of this investigation. Trial registration: DRKS00006755.

## References

Adam, M. 1991. Therapy of osteoarthritis: which effects have preparations of gelatin? *Therapiewoche*, **38**: 2456–2461.

Aumüller, G., Aust, G., and Doll, A. 2010. *Anatomie*. 2nd ed. Duale Reihe. Georg Thieme Verlag, Stuttgart.

Bello, A.E., and Oesser, S. 2006. Collagen hydrolysate for the treatment of osteoarthritis and other joint disorders: a review of the literature. *Curr. Med. Res. Opin.* **22**: 2221–2232. doi:10.1185/030079906X148373. PMID:17076983.

Benito-Ruiz, P., Camacho-Zambrano, M.M., Carrillo-Arcentales, J.N., Mestanza-Peralta, M.A., Vallejo-Flores, C.A., Vargas-López, S.V., et al. 2009. A randomized controlled trial on the efficacy and safety of a food ingredient, collagen hydrolysate, for improving joint comfort. *Int. J. Food Sci. Nutr.* **60**(S2): 99–113. doi:10.1080/09637480802498820. PMID:19212858.

Boling, M., Padua, D., Marshall, S., Guskiewicz, K., Pyne, S., and Beutler, A. 2010. Gender differences in the incidence and prevalence of patellofemoral pain syndrome. *Scand. J. Med. Sci. Sports*, **20**: 725–730. doi:10.1111/j.1600-0838.2009.00996.x. PMID:19765240.

Bruyère, O., Zegels, B., Leonori, L., Rabenda, V., Janssen, A., Bourges, C., and Reginster, J. 2012. Effect of collagen hydrolysate in articular pain: a 6-month randomized, double-blind, placebo controlled study. *Complement. Ther. Med.* **20**: 124–130. doi:10.1016/j.ctim.2011.12.007. PMID:22500661.

Butler, D.L., Noyes, F.R., and Grood, E.S. 1980. Ligamentous restraints to anterior-posterior drawer in the human knee. A biomechanical study. *J. Bone Joint Surg. Am.* **62**: 259–270. doi:10.2106/00004623-198062020-00013. PMID:7358757.

Carpenter, J., Peel, J., Carpenter, M., Lowndes, J., Angelopoulos, T., and Rippe, J.M. 2005. Effectiveness of a collagen hydrolysate-based supplement on joint pain, range of motion and muscle function in individuals with mild osteoarthritis of the knee: a randomized clinical trial. *Ann. Rheum. Dis.* **64**: 1544.

Clark, K.L., Sebastianelli, W., Flechsenhar, K.R., Aukermann, D.F., Meza, F., Millard, R.L., et al. 2008. 24-Week study on the use of collagen hydrolysate as a dietary supplement in athletes with activity-related joint pain. *Curr. Med. Res. Opin.* **24**: 1485–1496. doi:10.1185/030079908X291967. PMID:18416885.

Clemente, A. 2000. Enzymatic protein hydrolysates in human nutrition. *Trends Food Sci. Technol.* **11**: 254–262. doi:10.1016/S0924-2244(01)00007-3.

Dybka, K.A., Walczak, P. 2009. Collagen hydrolysates as a new diet supplement. *Hydrolyzaty kolagenu jako nowy suplement diety*.

European, Food, and Safety, Authority 2005. Opinion of the Scientific Panel on biological hazards (BIOHAZ) on the safety of collagen and a processing method for the production of collagen. *EFSA J.* **3**: 174. doi:10.2903/j.efsa.2005.174.

FDA (U.S. Food and Drug Administration). 2003. Chronological list of petitions and advisory opinions. URL: <http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0195-01-vol143.pdf> [date 31.07.2014]

Fichter, M., Körner, U., Schömburg, J., Jennings, L., Cole, A.A., and Mollenhauer, J. 2006. Collagen degradation products modulate matrix metalloproteinase expression in cultured articular chondrocytes. *J. Orthop. Res.* **24**: 63–70. doi:10.1002/jor.20001.

Flechsenhar, K., and Alf, D. 2005. Results of a postmarketing surveillance study of collagen hydrolysate CH-alpha. *Orthop. Prax.* **9**: 486–494.

Hartog, A., Cozijnsen, M., de Vrij, G., and Garssen, J. 2013. Collagen hydrolysate inhibits zymosan-induced inflammation. *Exp. Biol. Med.* **238**: 798–802. doi:10.1177/1535370213480740.

Iwai, K., Hasegawa, T., Taguchi, Y., Morimatsu, F., Sato, K., Nakamura, Y., et al. 2005. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *J. Agric. Food Chem.* **53**: 6531–6536. doi:10.1021/jf050206p. PMID:16076145.

Jennings, L., Wu, L., King, K.B., Hämmerle, H., Cs-Szabo, G., and Mollenhauer, J. 2001. The effects of collagen fragments on the extracellular matrix metabolism of bovine and human chondrocytes. *Connect. Tissue Res.* **42**: 71–86. doi:10.3109/03008200109014250. PMID:11696990.

Krug, E. 1979. Zur unterstützenden Therapie bei Osteo- und Chondropathien. [On supportive therapy for osteo- and chondropathy.] *Z. Für Erfahrungskheilkunde*, **11**: 930–938.

Kumar, S., Sugihara, F., Suzuki, K., Inoue, N., and Venkateswarathirukumara, S. 2015. A double-blind, placebo-controlled, randomised, clinical study on the effectiveness of collagen peptide on osteoarthritis. *J. Sci. Food Agric.* **95**: 702–707. doi:10.1002/jsfa.6752. PMID:24852756.

McAlindon, T.E., Nuite, M., Krishnan, N., Ruthazer, R., Price, L.L., Burstein, D., et al. 2011. Change in knee osteoarthritis cartilage detected by delayed gadolinium enhanced magnetic resonance imaging following treatment with collagen hydrolysate: a pilot randomized controlled trial. *Osteoarthritis. Cartil. OARS Osteoarthritis Res. Soc.* **19**: 399–405. doi:10.1016/j.joca.2011.01.001.

McAlindon, T., Bartnik, E., Ried, J.S., Teichert, L., and Herrmann, M. 2017. Determination of serum biomarkers in osteoarthritis patients: a previous interventional imaging study revisited. *J. Biomed. Res.* **31**: 1–6.

McMurray, T.P. 1942. The semilunar cartilages. *Br. J. Surg.* **29**: 407–414. doi:10.1002/bjs.18002911612.

Moskowitz, R.W. 2000. Role of collagen hydrolysate in bone and joint disease. *Semin. Arthritis Rheum.* **30**: 87–99. doi:10.1053/sarh.2000.9622. PMID:11071580.

Ng, K.W., Saliman, J.D., Lin, E.Y., Statman, L.Y., Kugler, L.E., Lo, S.B., et al. 2007. Culture duration modulates collagen hydrolysate-induced tissue remodeling in chondrocyte-seeded agarose hydrogels. *Ann. Biomed. Eng.* **35**: 1914–1923. doi:10.1007/s10439-007-9373-z. PMID:17721729.

Oberschelp, U. 1985. Individual therapy of osteoarthritis is possible. *Therapiewoche*, **35**: 5094–5097.

Oesser, S., and Seifert, J. 2003. Stimulation of type II collagen biosynthesis and secretion in bovine chondrocytes cultured with degraded collagen. *Cell Tissue Res.* **311**: 393–399. doi:10.1007/s00441-003-0702-8. PMID:12658447.

Oesser, S., Adam, M., Babel, W., and Seifert, J. 1999. Oral administration of (14)C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). *J. Nutr.* **129**: 1891–1895. PMID:10498764.

Ohara, H., Matsumoto, H., Ito, K., Iwai, K., and Sato, K. 2007. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J. Agric. Food Chem.* **55**: 1532–1535. doi:10.1021/jf062834s. PMID:17253720.

Piedras-Jorge, C., Meléndez-Moral, J.C., and Tomás-Miguel, J.M. 2010. Beneficios del ejercicio físico en población mayor institucionalizada. [Benefits of physical activity in nursing home residents.] *Rev. Esp. Geriatria Gerontol.* **45**: 131–135. [In Spanish.] doi:10.1016/j.regg.2009.10.012.

Research Randomizer. [WWW Document]. n.d. URL <https://www.randomizer.org/> [accessed 3.2.16].

Saito, M., Takenouchi, Y., Kunisaki, N., and Kimura, S. 2001. Complete primary structure of rainbow trout type I collagen consisting of  $\alpha 1(I)\alpha 2(I)\alpha 3(I)$  heterotrimers. *Eur. J. Biochem.* **268**: 2817–2827. doi:10.1046/j.1432-1327.2001.02160.x.

Schadow, S., Siebert, H.-C., Lochnit, G., Kordelle, J., Rickert, M., and Steinmeyer, J. 2013. Collagen metabolism of human osteoarthritic articular cartilage as modulated by bovine collagen hydrolysates. *PLoS ONE*, **8**: e53955. doi:10.1371/journal.pone.0053955. PMID:23342047.

Siebert, H.-C., Burg-Roderfeld, M., Eckert, T., Stötzel, S., Kirch, U., Diercks, T., et al. 2010. Interaction of the  $\alpha 2A$  domain of integrin with small collagen fragments. *Protein Cell*, **1**: 393–405. doi:10.1007/s13238-010-0038-6. PMID:21203951.

Steinmann, F. 1929. Referat über Meniskusverletzungen. *Schweiz. Med. Wochenschr.* **10**: 1355–1356.

Stötzel, S., Schurink, M., Wienk, H., Siebler, U., Burg-Roderfeld, M., Eckert, T., et al. 2012. Molecular organization of various collagen fragments as revealed

- by atomic force microscopy and diffusion-ordered NMR spectroscopy. *ChemPhysChem*. **13**: 3117–3125. doi:[10.1002/cphc.201200284](https://doi.org/10.1002/cphc.201200284).
- Walrand, S., Chiotelli, E., Noirt, F., Mwewa, S., and Lassel, T. 2008. Consumption of a functional fermented milk containing collagen hydrolysate improves the concentration of collagen-specific amino acids in plasma. *J. Agric. Food Chem.* **56**: 7790–7795. doi:[10.1021/jf800691f](https://doi.org/10.1021/jf800691f). PMID:[18707117](https://pubmed.ncbi.nlm.nih.gov/18707117/).
- Watanabe-Kamiyama, M., Shimizu, M., Kamiyama, S., Taguchi, Y., Sone, H., Morimatsu, F., et al. 2010. Absorption and effectiveness of orally administered low molecular weight collagen hydrolysate in rats. *J. Agric. Food Chem.* **58**: 835–841. doi:[10.1021/jf9031487](https://doi.org/10.1021/jf9031487). PMID:[19957932](https://pubmed.ncbi.nlm.nih.gov/19957932/).
- Witvrouw, E., Callaghan, M.J., Stefanik, J.J., Noehren, B., Bazett-Jones, D.M., Willson, J.D., et al. 2014. Patellofemoral pain: consensus statement from the 3rd International Patellofemoral Pain Research Retreat held in Vancouver, September 2013. *Br. J. Sports Med.* **48**: 411–414. doi:[10.1136/bjsports-2014-093450](https://doi.org/10.1136/bjsports-2014-093450). PMID:[24569145](https://pubmed.ncbi.nlm.nih.gov/24569145/).