NRC Research Press

ARTICLE

Effectiveness of collagen supplementation on pain scores in healthy individuals with self-reported knee pain: a randomized controlled trial

Coen C.W.G. Bongers, Dominique S.M. Ten Haaf, Milène Catoire, Bregina Kersten, Jeroen A. Wouters, Thijs M.H. Eijsvogels, and Maria T.E. Hopman

Abstract: The purpose of this study was to examine the effects of 12 weeks collagen peptide (CP) supplementation on knee pain and function in individuals with self-reported knee pain. Healthy physically active individuals (n = 167; aged 63 [interquartile range = 56–68] years) with self-reported knee pain received 10 g/day of CP or placebo for 12 weeks. Knee pain and function were measured with the Visual Analog Scale (VAS), the Lysholm questionnaire, and the Knee injury and Osteoarthritis Outcome Score (KOOS). Furthermore, we assessed changes in inflammatory, cartilage, and bone (bio)markers. Measurements were conducted at baseline and after 12 weeks of supplementation. Baseline VAS did not differ between CP and placebo (4.7 [2.5–6.1] vs. 4.7 [2.8–6.2], p = 0.50), whereas a similar decrease in VAS was observed after supplementation (–1.6 ± 2.4 vs. –1.9 ± 2.6, p = 0.42). The KOOS and Lysholm scores increased after supplementation in both groups (p values < 0.001), whereas the increase in the KOOS and Lysholm scores did not differ between groups (p = 0.28 and p = 0.76, respectively). Furthermore, CP did not impact inflammatory, cartilage, and bone (bio)markers (p values > 0.05). A reduced knee pain and improved knee function were observed following supplementation, but changes were similar between groups. This suggests that CP supplementation over a 12-week period does not reduce knee pain in healthy, active, middle-aged to elderly individuals.

Novelty

- CP supplementation over a 12-week period does not reduce knee pain in healthy, active, middle-aged to elderly individuals.
- CP supplementation over a 12-week period does not impact on inflammatory, cartilage, and bone (bio)markers in healthy, active, middle-aged to elderly individuals.

Key words: knee discomfort, collagen hydrolysate, cartilage, collagen synthesis, Lysholm questionnaire, KOOS questionnaire, inflammation, bone biomarkers.

Résumé : Le but de cette étude est d'examiner les effets de 12 semaines de supplémentation en peptide de collagène (« CP ») sur la douleur et la fonction du genou chez les personnes souffrant de douleur autodéclarée au genou. Les individus (n = 167) en bonne santé et physiquement actifs (63 [écart interquartile = 56–68] ans) présentant une douleur autodéclarée au genou reçoivent pendant 12 semaines 10 g/jour de CP ou un placebo. La douleur et la fonction du genou sont mesurées avec l'échelle visuelle analogique (« VAS »), les questionnaires de Lysholm et de KOOS (*Knee injury and Osteoarthritis Outcome Score*). De plus, nous évaluons les changements des (bio)marqueurs inflammatoires, cartilagineux et osseux. Les mesures sont effectuées au départ et après 12 semaines de supplémentation. Le score VAS initial ne diffère pas entre le CP et le placebo (4,7 [2,5–6,1] vs 4,7 [2,8–6,2], p = 0,50); toutefois, on note une diminution similaire du score VAS après la supplémentation (–1,6 ± 2,4 vs –1,9 ± 2,6, p = 0,42). Les scores KOOS et Lysholm augmentent après la supplémentation dans les deux groupes (valeurs p < 0,001), mais l'augmentation des scores KOOS et Lysholm ne diffère pas entre les groupes (p = 0,28 et p = 0,76, respectivement). De plus, le CP n'a pas d'impact sur les (bio)marqueurs inflammatoires, cartilagineux et osseux (valeurs de p > 0,05). Une réduction de la douleur au genou et une amélioration de la fonction du genou sont observées après la supplémentation, mais les changements sont similaires dans les deux groupes. Ces résultats suggèrent que la supplémentation en CP sur une période de 12 semaines ne réduit pas la douleur au genou chez les personnes d'âge moyen en bonne santé et les personnes âgées. [Traduit par la Rédaction]

Les nouveautés

- La supplémentation en CP sur une période de 12 semaines ne réduit pas la douleur au genou chez les personnes d'âge moyen et plus âgées, actives et en bonne santé.

Received 9 September 2019. Accepted 21 January 2020.

C.C.W.G. Bongers, D.S.M. Ten Haaf, B. Kersten, T.M.H. Eijsvogels, and M.T.E. Hopman. Radboud Institute for Health Sciences, Department of Physiology, Radboud University Medical Center, Nijmegen 6500HB, the Netherlands.
M. Catoire. Radboud Institute for Health Sciences, Department of Physiology, Radboud University Medical Center, Nijmegen 6500HB, the Netherlands; TNO, Expertise Group for Training and Performance Innovations, Soesterberg 3769ZG, the Netherlands.
J.A. Wouters. Sports Centre Papendal/Eat2Move, Arnhem 6816VD, the Netherlands.
Corresponding author: Thijs M.H. Eijsvogels (email: Thijs.Eijsvogels@radboudumc.nl).

- La supplémentation en CP sur une période de 12 semaines n'a pas d'impact sur les (bio)marqueurs inflammatoires, cartilagineux et osseux chez les personnes d'âge moyen et plus âgées, actives et en bonne santé.

Mots-clés : malaise au genou, hydrolysat de collagène, cartilage, synthèse de collagène, questionnaire de Lysholm, questionnaire de KOOS, inflammation, biomarqueurs osseux.

Introduction

Articular cartilage is important for the transmission of loads and to provide a smooth surface for low-friction joint movement (DuRaine et al. 2009; Sophia Fox et al. 2009). Lubrication of the cartilage surfaces is essential for a normal joint function (DuRaine et al. 2009). In individuals with articular cartilage degradation, the catabolic degradation exceeds the anabolic regeneration of articular cartilage by the chondrocytes (Goldring and Berenbaum 2004; Goldring and Goldring 2004; Mueller and Tuan 2011). This results in a limited ability for low-friction movements and consequently in the development of knee joint pain and discomfort.

In 2000, Moskowitz was one of the first to describe the potential beneficial effects of collagen peptides (CPs), also known as collagen hydrolysate, as a treatment for osteoarthritis and osteoporosis (Moskowitz 2000). Subsequently, a study by Oesser and Seifert was the first demonstrating that CPs are able to stimulate the biosynthesis of the extracellular matrix (ECM) of cartilage (Oesser and Seifert 2003), but this finding was not confirmed in preclinical studies (Schadow et al. 2013, 2017). Proteoglycans are the principal components of the ECM of articular cartilage and are critical for low-friction joint movements and the prevention of knee discomfort (Cohen et al. 1998; Iozzo and Murdoch 1996; Iozzo and Schaefer 2015; Sophia Fox et al. 2009). Interestingly, changes in proteoglycan content in knee cartilage have been found using magnetic resonance imaging measures among individuals taking CP supplements for 24 weeks (McAlindon et al. 2011). The use of CP supplements is therefore considered as a beneficial strategy to reduce knee pain and discomfort in patients with articular cartilage degradation (Clark et al. 2008; Zdzieblik et al. 2016). Furthermore, it has been suggested that various CP supplements differ in composition of collagen fragments and, therefore, differ in pharmacological efficacy on human synovial fibroblasts and cartilage (Schadow et al. 2013, 2017; Simons et al. 2018). In those studies, undigested CP was used in cell culture assays to determine its pharmacological efficacy, which is different from a physiological situation in which CP is modified during gastrointestinal passage by digestion enzymes and absorption processes (Sato 2017). Therefore, the use of digested CP supplements in cell culture assay studies is warranted to confirm the pharmacological efficacy of different CP supplements.

To our knowledge the effects of CP supplementation have only been examined in young athletes (Clark et al. 2008) or in patients with diagnosed osteoarthritis (Kumar et al. 2015; Lugo et al. 2016). Therefore, the aim of this study was to determine the effects of 12 weeks of CP supplementation on knee pain and knee function in healthy, active, middle-aged to elderly individuals with self-reported knee pain. Second, we examined the effects of CP supplementation on changes in inflammatory, cartilage, and bone (bio)markers. We hypothesized that 12 weeks of CP supplementation will reduce knee pain and improve knee function among active elderly. Furthermore, we expected a decrease in inflammatory, cartilage, and bone (bio)markers after 12 weeks of CP supplementation.

Materials and methods

Participants

Participants were recruited between 22 March and 8 April 2016. A total of 200 physically active participants (85 females and 115 males), aged between 50 and 75 years, volunteered to participate. All participants were in preparation for a multiple-day prolonged walking event (Four Days Marches, www.4daagse.nl) and walked ~30 km/week during the intervention period. Included participants had a self-reported knee pain score in daily life > 1, which was measured with the 100 mm Visual Analog Scale (VAS) (Hawker et al. 2011). Participants were excluded based on the following criteria: (*i*) systemic joint or muscle disease (i.e., rheumatoid arthritis), (*ii*) diabetes mellitus type 1 or 2, (*iii*) disease influencing the uptake of proteins (i.e., inflammatory bowel disease, Crohn's disease), (*iv*) recent knee surgery (<6 months), (*v*) statin usage, and (*vi*) use of dietary supplements for joint health (i.e., glucosamine or chondroitin). All participants gave written informed consent. The study was approved by a Medical Ethics Committee (NL56165.072.15), registered at a clinical trial register (Dutch Trial Register; NTR5825), and performed in accordance with the *Declaration of Helsinki*. Patients and the public were not involved in this study.

Study design

In this double-blind, placebo-controlled trial, participants were randomly allocated to either the intervention or placebo groups. An independent representative randomized the study participants by means of computer-generated random numbers with a block size of 10 in a 1:1 ratio. First, potential participants were screened by phone to check for eligibility to participate (inclusion and exclusion criteria, and knee pain score using the categorical Numeric Pain Rating Scale (NPRS; 0–10)). Eligible participants were invited for 2 study visits (baseline and after 12 weeks of supplementation). At baseline, anthropometric data were measured and participants completed knee pain (VAS and Knee injury and Osteoarthritis Outcome Score (KOOS)) and knee function (Lysholm) questionnaires. A venous blood sample was taken to assess inflammatory, cartilage, and bone (bio)markers. Subsequently, participants started with the daily intake of the CP or placebo supplement for 12 weeks. Participants had to complete a compliance questionnaire every week. After 12 weeks of supplementation the questionnaires were repeated and a venous blood sample was taken again.

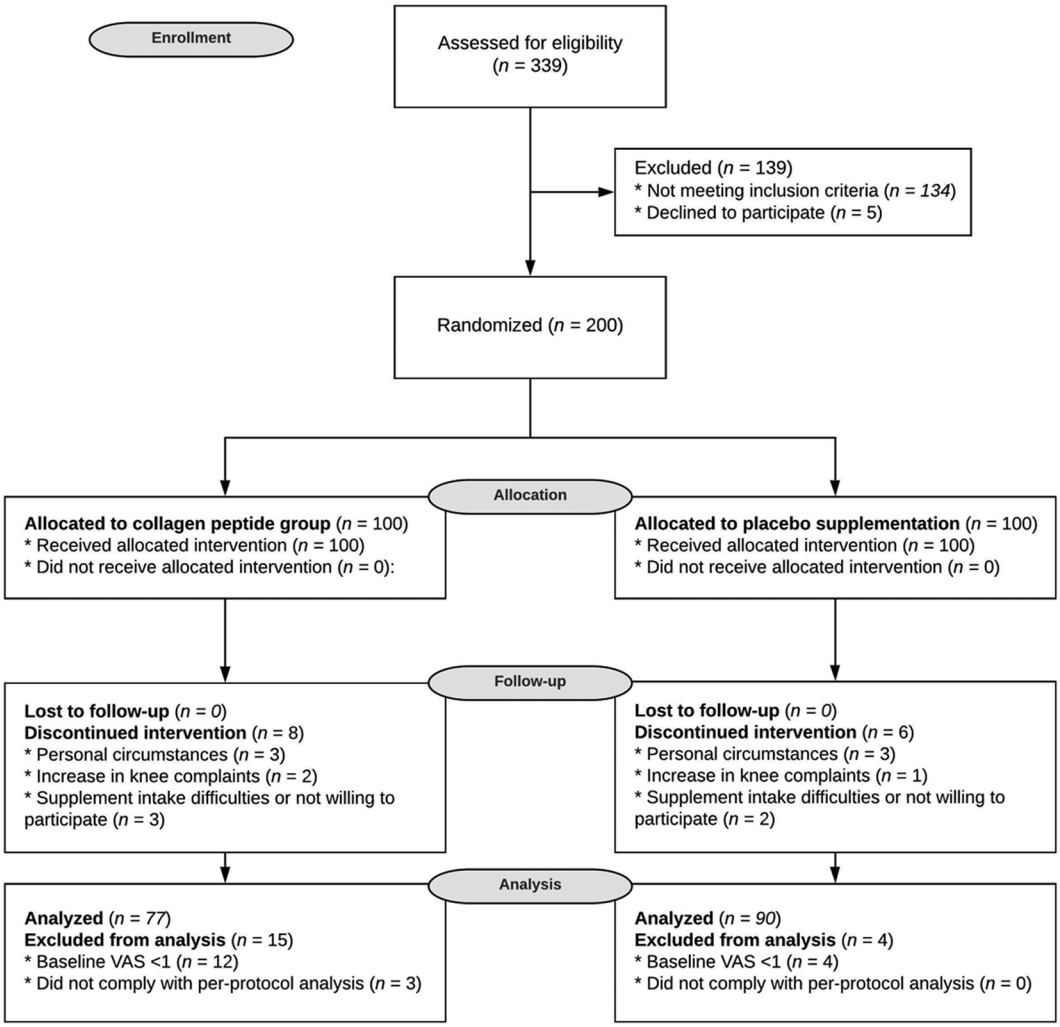
Intervention

Participants were randomly assigned to either a CP group ($n = 100$) that received a CP supplement (Peptan B2000, Rousselot, Gent, Belgium, molecular weight 2000 Da) for 10 g per day that was derived from bovine hide (hydrolyzed collagen; proline/hydroxyproline (23%), glycine (21%), glutamic acid (12%), arginine (8%), alanine (8%), essential amino acids (16%), and other amino acids (12%)) or a placebo group ($n = 100$) that received 10 g of maltodextrin on a daily basis. Peptan B2000 has proven efficacy to support joint health in both mice (Dar et al. 2017) and humans (Jiang et al. 2014), respectively, while in both studies a different Peptan B2000 batch was used. Moreover, participants in our study used CP supplements from a single batch. The supplement had to be dissolved in 100 mL of water and consumed for 12 weeks in combination with their habitual breakfast. The CP and placebo supplement were indistinguishable from each other in terms of color, taste, and viscosity. The research team was blinded for the randomization order and an independent representative was the only person able to break the randomization code.

Anthropometric measurements

Height and weight (Seca 888 scale, Hamburg, Germany) were measured and used to calculate the body mass index (BMI). Body

Fig. 1. Flowchart with overview of study participants. VAS, Visual Analog Scale.



fat percentage was calculated using a 4-point (biceps, triceps, subscapular, and sub-iliac) skinfold thickness measurement (Durnin and Womersley 1974). Furthermore, all participants completed the short questionnaire to assess health enhancing physical activity to determine their habitual physical activity level (Nicolaou et al. 2016). Handgrip strength of the dominant hand was measured using a hydraulic analogue hand dynamometer (Jamar, Jackson, Mich., USA). Participants were seated with their elbow flexed in a 90° angle position, and the dynamometer was adjusted to their individual hand size. Three measurements were performed, with 30 s of rest in between. Maximum strength in kilograms was used for analysis.

Knee pain and function questionnaires

The primary outcome of this study was the VAS, which is a widely accepted method for determining knee pain (Haefeli and Elfering 2006; Hawker et al. 2011). The VAS consists of a horizontal line of 100 mm in length, which is anchored by "no pain" (score of 0 mm) and "worst imaginable pain" (score of 100 mm) (Hawker et al. 2011). Participants were instructed to draw a vertical line on the VAS to indicate their level of knee pain. The Lysholm questionnaire was used to evaluate knee function based on instability, swelling, locking symptoms, pain, need for support, possibility of squatting and stair climbing, and the level of limb walking (Briggs et al. 2009). The cumulative Lysholm score was calculated and interpreted according the following knee function categories: <65, poor; 65–83, fair; 84–90, good; and 91–100, excellent (Briggs et al. 2009). The KOOS is a patient-reported outcome measure intended for elderly adults with knee injury and/or knee osteoarthritis, and can be used to monitor disease following pharmacological and other interventions (Collins et al. 2016; Roos et al. 1998). The KOOS questionnaire holds 5 subscales: symptoms, stiffness, pain, function in daily life, function in sports, and quality of life (Collins et al. 2016). Each subscale is scored separately from 0 (extreme knee problems) to 100 (no knee problems), and a total KOOS can be calculated based on the 5 subscales. The assessment of VAS, Lysholm, and KOOS were performed at baseline and after 12 weeks of supplementation. Furthermore, participants were instructed to score their level of knee pain on weekly basis using the NPRS (Hawker et al. 2011), a digitalized alternative of the VAS.

Cytokines and biomarkers

Nonfasted venous blood was drawn from the antecubital vein before and after the 12 weeks supplementation period, and serum samples were centrifuged (1200*g*/3000 rpm) and stored at −80 °C until analysis. Serum interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and C-reactive protein (CRP) were measured with the MSD multi-spot assay system (MSD; Meso Scale Discovery, Rockville, Md., USA) to examine inflammatory state (Pearle et al. 2007; Scheller et al. 2011; Villiger et al. 1992; Wojdasiewicz et al. 2014). In addition, C-terminal cross-linked telopeptide type II collagen (CTX-II) and procollagen II C-terminal propeptide (P2CP) were measured using an enzyme-linked immunosorbent assay (ELISA) (Hu CTX-II &

Table 1. Participant characteristics.

	All (n = 167)	CP (n = 77)	Placebo (n = 90)	p
Sex (male/female)	99/68	49/28	50/40	0.29
Age (y)*	63 [56–68]	65 [58–68]	61 [55–67]	0.036
Height (cm)*	173 [166–181]	174 [166–180]	173 [165–182]	0.85
Body mass (kg)	80.5±12.9	79.8±12.3	81.2±13.5	0.48
BMI (kg/m^2)*	26.4 [24.2–28.9]	26.4 [24.1–28.7]	26.1 [24.2–29.9]	0.50
Fat percentage (%)*	31.6 [26.2–38.5]	29.8 [24.9–37.8]	33.6 [27.4–39.2]	0.12
Handgrip strength (kg)*	38 [30–46]	40 [32–46]	38 [28–48]	0.39
Physical activity level (MET h/wk)*	140 [96–177]	134 [94–168]	146 [97–181]	0.41

Note: Data are presented as means ± SD or median [interquartile range]. An independent t test or Mann–Whitney U test was used to examine differences between groups. Sex differences were examined using a χ^2 test. BMI, body mass index; CP, collagen peptide.

*Not normally distributed data.

PIICP kit, Cloud-Clone Corp, China) and represented cartilage breakdown and cartilage formation, respectively (Conrozier et al. 2008; Fraser et al. 2003; Rotterud et al. 2014). Furthermore, Carboxy-terminal telopeptides (CTX) and procollagen I intact N-terminal (PINP) were measured with an electro-chemiluminescence immunoassay (ECLIA) (β-crosslaps & total PINP, Roche Diagnostics International Ltd, Switzerland) and represented bone resorption and bone formation, respectively (Luftner et al. 2005; Lumachi et al. 2013). Analysis were performed by trained technicians using standard operating procedures on a single day using the same calibration and set-up to minimize variation.

Statistical analysis

A per protocol analysis was used including only the participants that completed all study procedures for the primary outcome. Statistical analysis was performed using SPSS (version 25; IBM SPSS, Armonk, N.Y., USA), in which the level of significance was set at $p < 0.05$. Normality of the data was examined using a Shapiro–Wilk test. Normally distributed data were reported as means ± standard deviation, whereas non-Gaussian distributed data were presented as median [interquartile range (IQR)] and tested with nonparametric equivalent statistical tests. Furthermore, data were presented with 95% confidence intervals (CI) as well. A χ^2 test was used to examine differences in sex distribution between groups. An independent Student's t test or a Mann–Whitney U test was used to examine differences in participant characteristics between groups. Supplement compliance was calculated as a percentage of the number of supplements taken divided by the total number of available supplements during the study. Because the majority of data was non-Gaussian distributed, we used a paired Student's t test or a Wilcoxon signed-rank test to assess differences in baseline characteristics and knee outcome parameters (VAS, KOOS, and Lysholm) between baseline and week 12 in both groups. Subsequently, we calculated differences (Δ) in knee pain, knee function, inflammatory markers, and bone and cartilage biomarkers between baseline and week 12, and used an independent Student's t test or a Mann–Whitney U test to assess differences between the CP versus placebo groups. Linear mixed model analysis was used to determine whether time-dependent changes in knee pain (NPRS) differed between groups during the supplementation period. A post hoc Bonferroni correction was applied to correct for multiple comparisons.

Results

Participants

Although all participants scored their level of knee pain >1 upon study enrollment (NPRS during telephone screening), a total of 16 participants (CP: n = 12; placebo: n = 4) reported a VAS < 1 at baseline and were, therefore, excluded from further analysis. Furthermore, 17 participants dropped-out during the supplementation period, resulting in a total of 167 participants (CP: n = 77; placebo: n = 90) available for statistical analyses (Fig. 1). Supplement intake difficulties (gastrointestinal complaints, intake difficulties, or a smelly breath) were reported in 3 participants, while no other adverse events were reported. Participants in the CP group were significantly older compared with the placebo group (65 [IQR = 58–68, 95% CI = 62–65] years versus 61 [IQR = 55–67, 95% CI = 60–63] years, p = 0.036). Sex, BMI, and physical activity characteristics did not differ between groups (all p values > 0.05, Table 1). Supplement compliance was high and did not differ between groups (CP = 99.6% ± 1.2%, 95% CI = 99.3–99.9 vs. placebo = 99.4% ± 1.5%, 95% CI = 99.1–99.7, p = 0.29).

Knee pain and function

VAS

Baseline VAS was 4.7 [IQR = 2.5–6.1, 95% CI = 3.9–4.9] for the CP group and 4.7 [IQR = 2.8–6.2, 95% CI = 4.2–5.0] for the placebo group, and did not differ between groups (p = 0.50, Fig. 2). We found a decrease in VAS in both groups after 12 weeks of supplementation (both $p < 0.001$), while the magnitude of the decrease (ΔCP = –1.6 ± 2.4, 95% CI = –2.1 to –1.1; ΔPlacebo = –1.9 ± 2.6, 95% CI = –2.5 to –1.4) did not differ between groups (p = 0.42). Similarly, a gradual decline in NPRS scores was found during 12 weeks of follow-up ($p < 0.001$), but the decline did not differ between groups (p = 0.20, Supplemental Fig. S1[1]).

KOOS

Baseline KOOS score did not differ between the CP (302 ± 68, 95% CI = 287–317) and placebo groups (308 ± 64, 95% CI = 294–322, p = 0.55, Fig. 3A). An increased total KOOS score was found after 12 weeks of supplementation in both groups (ΔCP = 41 ± 56, 95% CI = 29–54; ΔPlacebo = 31 ± 68, 95% CI = 16–45, both $p < 0.001$), whereas the increase did not differ between groups (p = 0.28).

Lysholm

We found a comparable baseline Lysholm score in the CP group (IQR = 66 [56–71, 95% CI = 61–67]) and the placebo group (68 [IQR = 60–76, 95% CI = 65–70], p = 0.07, Fig. 3B). Both groups demonstrated an increased Lysholm score after 12 weeks of supplementation (both $p \leq 0.001$), whereas the increase did not differ between groups (ΔCP = 5 [IQR = –2 to 14, 95% CI = 3–8] and ΔPlacebo = 5 [IQR = –3 to 16, 95% CI = 3–9], p = 0.76).

Blood markers

Inflammatory markers

No baseline differences in serum IL-6, TNF-α, MCP1, and CRP were found between groups (p = 0.86, p = 0.28, p = 0.55, p = 0.80, respectively). Moreover, no changes in inflammatory markers

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/apnm-2019-0654.

Fig. 2. Knee pain measured with the Visual Analog Scale (VAS) at baseline and week 12 and the response to supplementation (Δ = week 12 – baseline) for the collagen peptide (CP; white bars) and placebo group (grey bars). Data are presented as median [interquartile range] (n = 167). A decrease in VAS was found in both groups, while the magnitude of the decrease did not differ between groups.

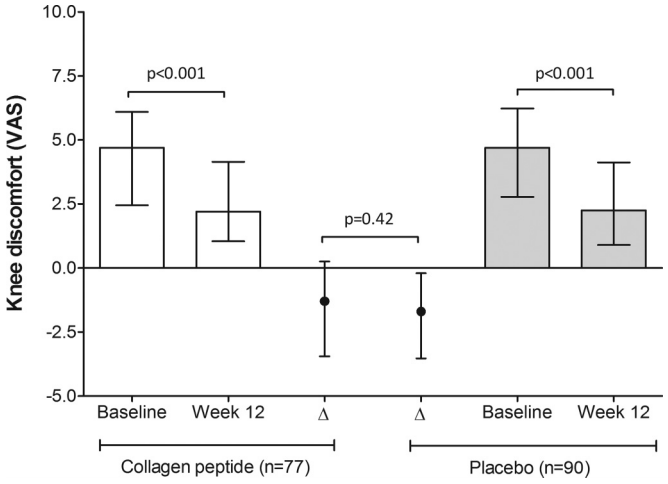
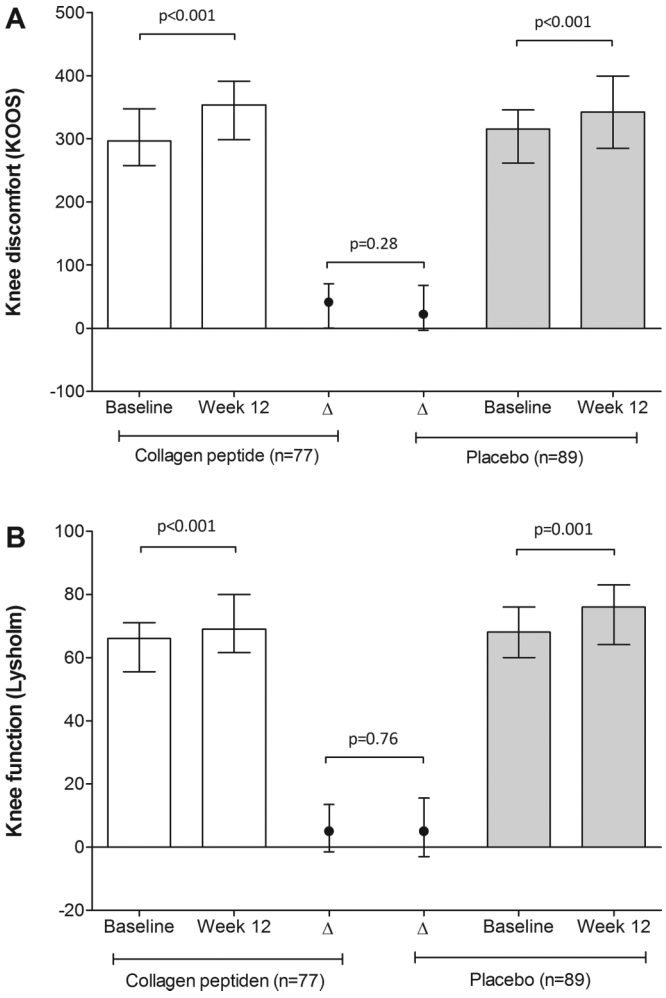


Fig. 3. Knee function measured with the Knee injury and Osteoarthritis Outcome Score (KOOS; A) and Lysholm questionnaire (B) at baseline and week 12 and the response to supplementation (Δ = week 12 – baseline) for the collagen peptide (CP; white bars) and placebo group (grey bars). Data are presented as median [interquartile range] (n = 166). An increase in KOOS and Lysholm score was found in both groups, while the magnitude of the increase did not differ between groups.



were found after supplementation in both groups (all $p > 0.05$), except for an increased IL-6 concentration in the placebo group (p = 0.012, Table 2).

Cartilage biomarkers

Baseline CTX-II and P2CP levels did not differ between both groups (p = 0.27 and p = 0.72, respectively). After 12 weeks of supplementation no change in CTX-II concentration was found in both groups (p = 0.41 and p = 0.65 for the CP and placebo groups, respectively), whereas the P2CP concentration was increased in the CP (p = 0.013) and placebo groups (p = 0.032, Table 2). Moreover, the increase in P2CP concentration did not differ between the CP (2.8 [IQR = –3.4–10.3, 95% CI = 0.7–6.0] μg/mL) and placebo groups (4.6 [IQR = –3.8–9.9, 95% CI = –1.3–4.4] μg/mL, p = 0.94, Table 2).

Bone biomarkers

Baseline CTX and PINP concentrations did not differ between both groups (p = 0.56 and p = 0.92, respectively). We found increased CTX levels after 12 weeks of supplementation in both groups (p = 0.037 and p = 0.023 for the CP and placebo group, respectively), whereas no differences in PINP concentration were found (p = 0.71 and p = 0.94, Table 2). Moreover, the increase in CTX concentration did not differ between the CP (0.022 [IQR = –0.029 to 0.070, 95% CI = –0.004 to 0.045] ng/mL) and placebo group (0.012 [IQR = –0.034 to 0.075, 95% CI = 0.002–0.040] ng/mL, p = 0.94, Table 2).

Discussion

In this double-blind, randomized, placebo-controlled trial, we examined the effects of 12 weeks of CP supplementation on knee pain and function in healthy, physically active, middle-aged to elderly individuals with self-reported knee pain. A reduced knee pain and improved knee function were found after 12 weeks of supplementation, but changes over time did not differ between groups. Moreover, in a study by Wandel et al., a difference in VAS score of 0.9 between measurements was defined as clinically relevant (Wandel et al. 2010), which suggest that the change in VAS after 12 weeks of supplementation (CP = –1.6 ± 2.4 and ΔPlacebo = –1.9 ± 2.6) was not only not statistically different (p = 0.42), but also not a clinically relevant difference between groups. Furthermore, we did not find any difference in inflammatory, cartilage, and bone (bio)markers after 12 weeks of supplementation between both groups. The absence of a superior effect in the CP group suggest that 12-weeks of CP supplementation did not contribute to reductions in knee joint pain in healthy, physically active, middle-aged to elderly individuals.

The main finding of our study was that we did not find a superior effect of 12 weeks of CP compared with placebo supplementation on knee pain and knee function. This is in contrast to most (Bruyere et al. 2012; Kumar et al. 2015), but not all (Lugo et al. 2013), previous studies. Bruyere and colleagues demonstrated that 6 months of collagen hydrolysate supplementation, in participants with joint pain (VAS > 3.0) at different joints (hip, knee, elbow, shoulder, hand, or/and lumbar spine) without diagnosed osteoarthritis, resulted in a greater proportion of participants with a clinical decrement in VAS (≥20%) compared with the placebo group (Bruyere et al. 2012). Our study demonstrated that a similar proportion of participants in the CP and placebo group (~60% vs. ~67%, respectively) demonstrated a decreased VAS ≥ 20%. Kumar et al. demonstrated in participants with diagnosed osteoarthritis and a baseline VAS > 4.0 (average VAS 6.3 ± 1.1 and 6.6 ± 1.2) that 13 weeks of CP supplementation derived from either pork skin or bovine bone resulted in an ~51% and ~58% decrease in VAS, respectively (Kumar et al. 2015). In contrast, we included

Table 2. Within- and between-group differences in blood markers.

	Collagen peptide			Placebo			Change during 12 weeks of supplementation		
	Baseline	Week 12	*p*	Baseline	Week 12	*p*	ΔCP	ΔPlacebo	*p*
IL-6 (pg/mL)	0.6 [0.4 to 0.8]	0.6 [0.5 to 0.9]	0.11	0.5 [0.4 to 0.8]	0.7 [0.5 to 0.9]	0.012	0.05 [−0.14 to 0.21]	0.07 [−0.09 to 0.25]	0.56
TNF-α (pg/mL)	1.9 [1.6 to 2.4]	1.9 [1.6 to 2.4]	0.85	1.9 [1.5 to 2.2]	1.9 [1.5 to 2.3]	0.71	0.01 [−0.27 to 0.24]	−0.09 [−0.23 to 0.25]	0.90
MCP-1 (pg/mL)	369 [300 to 457]	366 [311 to 453]	0.68	356 [289 to 438]	371 [317 to 433]	0.50	−1.8 [−44.2 to 40.9]	4.8 [−48.7 to 58.5]	0.45
CRP (μg/mL)	1.9 [1.1 to 4.2]	1.7 [0.8 to 3.2]	0.09	2.1 [1.0 to 3.8]	1.8 [0.9 to 3.5]	0.52	−0.1 [−1.7 to 0.5]	−0.1 [−1.1 to 0.6]	0.35
CTX-II (pg/mL)	421 [346 to 527]	434 [355 to 528]	0.41	419 [333 to 499]	412 [354 to 511]	0.65	3.7 [−26.5 to 45.1]	1.0 [−45.3 to 53.9]	0.83
P2CP (ng/mL)	20.8 [14.8 to 28.5]	24.3 [16.3 to 31.1]	0.013	21.7 [15.6 to 28.2]	25.5 [16.9 to 30.0]	0.032	2.8 [−3.4 to 10.3]	4.6 [−3.8 to 9.9]	0.94
CTX (ng/mL)	0.2 [0.1 to 0.3]	0.2 [0.2 to 0.3]	0.037	0.2 [0.1 to 0.3]	0.2 [0.2 to 0.3]	0.023	0.02 [−0.03 to 0.07]	0.01 [−0.03 to 0.08]	0.94
PINP (ng/mL)	42.5 [34.5 to 55.4]	42.4 [34.4 to 56.3]	0.71	43.2 [34.0 to 55.6]	42.5 [34.3 to 56.3]	0.94	1.1 [−4.6 to 4.5]	0.2 [−5.4 to 5.5]	0.92

Note: Data are presented as median [interquartile range]. Inflammatory, bone, and cartilage (bio)markers at baseline and after 12 weeks of supplementation. A nonparametric Wilcoxon signed-rank test was used to assess differences within groups, whilst a Mann–Whitney *U* test was used to assess differences between groups. CTX, carboxy-terminal telopeptides; CTX-II, C-terminal cross-linked telopeptide type II collagen; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein-1; P2CP, procollagen II C-terminal propeptide; PINP, procollagen I intact N-terminal; TNF-α, tumor necrosis factor alpha.

participants with a VAS > 1.0 for knee pain (average VAS 4.7 [IQR = 2.6–6.2, 95% CI = 4.2–4.8]) and did not find an effect of CP supplementation. Our study population consisted of 43 participants (n = 19 and n = 24 in CP and placebo, respectively) with a baseline VAS > 6.0. Within this subgroup of participants we did not find differences in VAS between groups, suggesting that the lower baseline VAS in our study did not explain the lack of difference between the CP and placebo groups. Although baseline VAS did not seem to influence the results, participants with diagnosed osteoarthritis have been excluded from participation in our study and therefore only relatively mild cases might have been included. Participants' Lysholm score at baseline was 67 [IQR = 58–74, 95% CI = 64–68], which corresponds to a fair knee function (Briggs et al. 2009). Therefore, knee complaints and associated decrements in knee function of our participants may not have been severe enough to find a beneficial effect of CP supplementation.

Another potential explanation for the decrease in knee pain and improvement in knee function outcomes in both the CP and the placebo group could be the presence of a placebo effect in both groups. Zhang and colleagues demonstrated an overall estimate of the effect size for pain relief of 0.51 (95% CI = 0.46–0.55) in studies focusing on osteoarthritis and pain (Zhang et al. 2008). Moreover, in 1955 Beecher combined data from placebo groups of 15 studies and demonstrated that placebos on average led to a 35% improvement in outcomes (Beecher 1955). Average improvement after 12 weeks of CP or placebo supplementation in our study was ~36% and ~53%, respectively. Therefore, the decrease in knee pain in both groups could mainly be attributed to a placebo effect. Moreover, the presence of this large placebo effect in both groups made it harder to find a significant difference between groups.

Additionally, in the present study a supplementation duration of 12 weeks was used, while previous studies with a positive outcome used a supplementation period of 24 weeks or longer (Bruyere et al. 2012; Clark et al. 2008; Lugo et al. 2016). This may suggest that long-term supplementation is needed to induce beneficial effects on knee pain, even more so in elderly who may have knee complaints for a long time already. However, 26% of the participants had a VAS lower than the exclusion threshold (VAS < 1) after 12 weeks of supplementation. These findings suggest that elongation of the supplementation period would probably not affect the results within our cohort of middle-aged to elderly physically active individuals.

We did not find a difference in inflammatory markers and bone and cartilage biomarkers between the CP and placebo group, which suggests that CP supplementation did not reduce the inflammatory status and did not stimulate bone formation or the synthesis of cartilage. This finding is in line with previous preclinical data that observed that the use of collagen hydrolysates does not stimulate type II collagen biosynthesis in human articular cartilage (Schadow et al. 2013, 2017). In our study, baseline concentrations of the inflammatory markers were all within the reference values and did not show evidence of whole-body inflammation (Todd et al. 2013). We did find an increased IL-6 concentration in the placebo group after 12 weeks of supplementation, while this increase was absent in the CP group. The authors are not able to explain the increased IL-6 concentration, and the increment is not supported by other inflammatory markers (i.e., TNF-α and CRP). Furthermore, the IL-6 concentration in the placebo group post-supplementation (0.7 [IQR = 0.5–0.9, 95% CI = 0.7–1.4]) was still within the upper 95th percentile reference limit of 4.45 pg/mL (Todd et al. 2013). The elevated levels of CTX and P2CP after 12 weeks of supplementation seem not to be the result of CP supplementation since we did not find a difference between both groups.

Strengths and limitations

A strength of the study is the design (double-blind, randomized, and placebo-controlled) and the inclusion of a large study population. Furthermore, the supplement compliance of the participants was very high (99.5% ± 1.3%, 95% CI = 99.3–99.7). However, there are some limitations that should be taken into account. First, we included participants with self-reported knee pain without diagnosis of osteoarthritis. Since no X-rays were obtained it is not certain that the participants had no osteoarthritis. Symptoms of arthritis can fluctuate over time, which could have led to a large heterogeneity within our study population. We tried to minimize the heterogeneity by excluding participants with specific medical conditions or supplement/medication intake. Moreover, all participants were in preparation for a multiple-day prolonged exercise event, which might suggest that our study population is a relatively active and healthy population, and is probably not representative for the general population with knee complaints. Furthermore, we randomly allocated participants to the CP and placebo group. Second, a total of 33 participants dropped out of the study (i.e., 33 out of 200, ~17%), whereas some (3 in total and 2 in the CP group) of these participants withdrew because of increasing knee pain. Moreover, significantly more dropouts were observed in the CP group (n = 23) compared with the placebo group (n = 10, p = 0.013). However, the difference in dropout rate can mainly (n = 16 out of 33, 50%) be attributed to a baseline VAS < 1, which makes it unlikely that dropout was influenced by the use of the CP supplement. As a consequence of the high dropout rate, the total number of participants in CP group (n = 77) was lower than the number of participants needed according to the sample size calculation (n = 86). However, based on current results we did not expect a difference when more participants were included. Although we did instruct participants to avoid using analgesics prior to the baseline measurements, we did not provide specific instructions for analgesics and/or NSAID use during the supplementation period. As a result, a total of 22 participants used analgesics (primarily paracetamol; n = 15) on the day of the post-

supplementation measurements, which may have influenced our findings. However, when we exclude these participants in a sensitivity analyses, similar outcomes were observed (change in VAS for knee pain after 12 weeks of supplementation was –1.7 ± 2.3 and –2.1 ± 2.5 for CP and control groups, respectively, $p = 0.33$). As the use of analgesics did not impact our study findings, we decided to keep the participants who used analgesics in our analysis. Furthermore, we did not monitor the participants' food intake throughout the study. Since CP is a hydrolyzed form of gelatin (Liu et al. 2015), it is possible, but quite unlikely, that a high CP consumption in daily life (desserts, bakery products, gummy candy) affects the outcomes of the study. Furthermore, such bias may have affected the CP and placebo group equally.

Conclusion

In conclusion, 12 weeks of CP and placebo supplementation resulted in a similar reduction in knee pain and improvement of knee function. The absence of a superior effect of CP suggest that CP supplementation over a 12-week period does not reduce knee joint pain in healthy, physically active, middle-aged to elderly individuals with self-reported knee pain.

Conflicts of interest statement

The work of T.M.H. Eijsvogels is supported by a European Commission Horizon 2020 grant (Marie Sklodowska-Curie Fellowship 655502) and the work by J.A. Wouters is supported by the Province of Gelderland (PS2014-49). Furthermore, this investigator-initiated study (Radboudumc) was supported by Rousselot (Gent, Belgium), in which the complete study, including research design, data collection, data analysis, was performed by the research team and not influenced by our funding partner. All other authors have nothing to declare.

Acknowledgements

We recognize the excellent help of the organization of the Nijmegen Four Days Marches. Furthermore, we want to thank the Wageningen University & Research (WUR) and the Leiden University Medical Center (LUMC) for their assistance with the blood sample analysis. The authors greatly acknowledge the enthusiasm and dedication of the participants in this study. Furthermore, the practical assistance of colleagues was greatly appreciated.

References

Beecher, H.K. 1955. The powerful placebo. J. Am. Med. Assoc. **159**(17): 1602–1606. doi:10.1001/jama.1955.02960340022006. PMID:13271123.

Briggs, K.K., Lysholm, J., Tegner, Y., Rodkey, W.G., Kocher, M.S., and Steadman, J.R. 2009. The reliability, validity, and responsiveness of the Lysholm score and Tegner activity scale for anterior cruciate ligament injuries of the knee: 25 years later. Am. J. Sports Med. **37**(5): 890–897. doi:10.1177/0363546508330143. PMID:19261899.

Bruyere, O., Zegels, B., Leonori, L., Rabenda, V., Janssen, A., Bourges, C., and Reginster, J.Y. 2012. Effect of collagen hydrolysate in articular pain: a 6-month randomized, double-blind, placebo controlled study. Complement. Ther. Med. **20**(3): 124–130. doi:10.1016/j.ctim.2011.12.007. PMID:22500661.

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**(5): 1485–1496. doi:10.1185/030079908X291967. PMID:18416885.

Cohen, N.P., Foster, R.J., and Mow, V.C. 1998. Composition and dynamics of articular cartilage: structure, function, and maintaining healthy state. J. Orthop. Sports Phys. Ther. **28**(4): 203–215. doi:10.2519/jospt.1998.28.4.203. PMID:9785256.

Collins, N.J., Prinsen, C.A., Christensen, R., Bartels, E.M., Terwee, C.B., and Roos, E.M. 2016. Knee Injury and Osteoarthritis Outcome Score (KOOS): systematic review and meta-analysis of measurement properties. Osteoarthritis Cartilage, **24**(8): 1317–1329. doi:10.1016/j.joca.2016.03.010. PMID:27012756.

Conrozier, T., Poole, A.R., Ferrand, F., Mathieu, P., Vincent, F., Piperno, M., et al. 2008. Serum concentrations of type II collagen biomarkers (C2C, C1, 2C and CPII) suggest different pathophysiologies in patients with hip osteoarthritis. Clin. Exp. Rheumatol. **26**(3): 430–435. PMID:18578964.

Dar, Q.A., Schott, E.M., Catheline, S.E., Maynard, R.D., Liu, Z.Y., Kamal, F., et al. 2017. Daily oral consumption of hydrolyzed type 1 collagen is chondroprotective and anti-inflammatory in murine posttraumatic osteoarthritis. PLoS One, **12**(4): e0174705. doi:10.1371/journal.pone.0174705. PMID:28384173.

DuRaine, G., Neu, C.P., Chan, S.M., Komvopoulos, K., June, R.K., and Reddi, A.H. 2009. Regulation of the friction coefficient of articular cartilage by TGF-beta1 and IL-1beta. J. Orthop. Res. **27**(2): 249–256. doi:10.1002/jor.20713. PMID:18683879.

Durnin, J.V., and Womersley, J. 1974. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br. J. Nutr. **32**(1): 77–97. doi:10.1079/BJN19740060. PMID:4843734.

Fraser, A., Fearon, U., Billinghurst, R.C., Ionescu, M., Reece, R., Barwick, T., et al. 2003. Turnover of type II collagen and aggrecan in cartilage matrix at the onset of inflammatory arthritis in humans: relationship to mediators of systemic and local inflammation. Arthritis Rheum. **48**(11): 3085–3095. doi:10.1002/art.11331. PMID:14613270.

Goldring, M.B., and Berenbaum, F. 2004. The regulation of chondrocyte function by proinflammatory mediators: prostaglandins and nitric oxide. Clin. Orthop. Relat. Res. **427**(Suppl.): S37–S46. doi:10.1097/01.blo.0000144484.69656.e4. PMID:15480072.

Goldring, S.R., and Goldring, M.B. 2004. The role of cytokines in cartilage matrix degeneration in osteoarthritis. Clin. Orthop. Relat. Res. **427**(Suppl.): S27–S36. doi:10.1097/01.blo.0000144854.66565.8f. PMID:15480070.

Haefeli, M., and Elfering, A. 2006. Pain assessment. Eur. Spine J. **15**(Suppl. 1): S17–S24. doi:10.1007/s00586-005-1044-x. PMID:16320034.

Hawker, G.A., Mian, S., Kendzerska, T., and French, M. 2011. Measures of Adult Pain Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). Arthrit. Care Res. **63**(S11): S240–S252. doi:10.1002/acr.20543. PMID:22588748.

Iozzo, R.V., and Murdoch, A.D. 1996. Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. FASEB J. **10**(5): 598–614. doi:10.1096/fasebj.10.5.8621059. PMID:8621059.

Iozzo, R.V., and Schaefer, L. 2015. Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. Matrix Biol. **42**: 11–55. doi:10.1016/j.matbio.2015.02.003. PMID:25701227.

Jiang, J.X., Yu, S., Huang, Q.R., Zhang, X.L., Zhang, C.Q., Zhou, J.L., and Prawitt, J. 2014. Collagen peptides improve knee osteoarthritis in elderly women A 6-month randomized, double-blind, placebo-controlled study. Agro. Food Ind. Hi Tec. **25**(2): 19–23.

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**(4): 702–707. doi:10.1002/jsfa.6752. PMID:24852756.

Liu, D.S., Nikoo, M., Boran, G., Zhou, P., and Regenstein, J.M. 2015. Collagen and Gelatin. Annu. Rev. Food Sci. Techonol. **6**: 527–557. doi:10.1146/annurev-food-031414-111800. PMID:25884286.

Luftner, D., Jozereau, D., Schildhauer, S., Geppert, R., Muller, C., Fiolka, G., et al. 2005. PINP as serum marker of metastatic spread to the bone in breast cancer patients. Anticancer Res. **25**(3A): 1491–1499. PMID:16033050.

Lugo, J.P., Saiyed, Z.M., Lau, F.C., Molina, J.P., Pakdaman, M.N., Shamie, A.N., and Udani, J.K. 2013. Undenatured type II collagen (UC-II(R®)) for joint support: a randomized, double-blind, placebo-controlled study in healthy volunteers. J. Int. Soc. Sports Nutr. **10**(1): 48. doi:10.1186/1550-2783-10-48. PMID:24153020.

Lugo, J.P., Saiyed, Z.M., and Lane, N.E. 2016. Efficacy and tolerability of an undenatured type II collagen supplement in modulating knee osteoarthritis symptoms: a multicenter randomized, double-blind, placebo-controlled study. Nutr. J. **15**: 14. doi:10.1186/s12937-016-0130-8. PMID:26822714.

Lumachi, F., Santeufemia, D.A., Del Conte, A., Mazza, F., Tozzoli, R., Chiara, G.B., and Basso, S.M. 2013. Carboxy-terminal Telopeptide (CTX) and Amino-terminal Propeptide (PINP) of Type I collagen as markers of bone metastases in patients with non-small cell lung cancer. Anticancer Res. **33**(6): 2593–2596. PMID:23749913.

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 Cartilage, **19**(4): 399–405. doi:10.1016/j.joca.2011.01.001. PMID:21251991.

Moskowitz, R.W. 2000. Role of collagen hydrolysate in bone and joint disease. Semin. Arthritis Rheum. **30**(2): 87–99. doi:10.1053/sarh.2000.9622. PMID:11071580.

Mueller, M.B., and Tuan, R.S. 2011. Anabolic/Catabolic balance in pathogenesis of osteoarthritis: identifying molecular targets. PM&R, **3**(6 Suppl. 1): S3–S11. doi:10.1016/j.pmrj.2011.05.009. PMID:21703577.

Nicolaou, M., Gademan, M.G.J., Snijder, M.B., Engelbert, R.H.H., Dijkshoorn, H., Terwee, C.B., and Stronks, K. 2016. Validation of the SQUASH physical activity questionnaire in a multi-ethnic population: the HELIUS study. PLoS ONE, **11**(8): e0161066. doi:10.1371/journal.pone.0161066. PMID:27575490.

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**(3): 393–399. doi:10.1007/s00441-003-0702-8. PMID:12658447.

Pearle, A.D., Scanzello, C.R., George, S., Mandl, L.A., DiCarlo, E.F., Peterson, M., et al. 2007. Elevated high-sensitivity C-reactive protein levels are associated

with local inflammatory findings in patients with osteoarthritis. Osteoarthritis Cartilage, **15**(5): 516–523. doi:10.1016/j.joca.2006.10.010. PMID:17157039.

Roos, E.M., Roos, H.P., Lohmander, L.S., Ekdahl, C., and Beynnon, B.D. 1998. Knee injury and osteoarthritis outcome score (KOOS)–development of a self-administered outcome measure. J. Orthop. Sports Phys. Ther. **28**(2): 88–96. doi:10.2519/jospt.1998.28.2.88. PMID:9699158.

Rotterud, J.H., Reinholt, F.P., Beckstrom, K.J., Risberg, M.A., and Aroen, A. 2014. Relationship between CTX-II and patient characteristics, patient-reported outcome, muscle strength, and rehabilitation in patients with a focal cartilage lesion of the knee: a prospective exploratory cohort study of 48 patients. BMC Musculoskelet. Disord. **15**: 99. doi:10.1186/1471-2474-15-99. PMID:24661577.

Sato, K. 2017. The presence of food-derived collagen peptides in human body-structure and biological activity. Food Funct. **8**(12): 4325–4330. doi:10.1039/c7fo01275f. PMID:29114654.

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**(1): e53955. doi:10.1371/journal.pone.0053955. PMID:23342047.

Schadow, S., Simons, V.S., Lochnit, G., Kordelle, J., Gazova, Z., Siebert, H.C., et al. 2017. Metabolic response of human osteoarthritic cartilage to biochemically characterized collagen hydrolysates. Int. J. Mol. Sci. **18**(1): E207. doi:10.3390/ijms18010207. PMID:28117674.

Scheller, J., Chalaris, A., Schmidt-Arras, D., and Rose-John, S. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim. Biophys. Acta, **1813**(5): 878–888. doi:10.1016/j.bbamcr.2011.01.034. PMID:21296109.

Simons, V.S., Lochnit, G., Wilhelm, J., Ishaque, B., Rickert, M., and Steinmeyer, J. 2018. Comparative analysis of peptide composition and bioactivity of different collagen hydrolysate batches on human osteoarthritic synoviocytes. Sci. Rep. **8**(1): 17733. doi:10.1038/s41598-018-36046-3. PMID:30531866.

Sophia Fox, A.J., Bedi, A., and Rodeo, S.A. 2009. The basic science of articular cartilage: structure, composition, and function. Sports Health, **1**(6): 461–468. doi:10.1177/1941738109350438. PMID:23015907.

Todd, J., Simpson, P., Estis, J., Torres, V., and Wub, A.H. 2013. Reference range and short- and long-term biological variation of interleukin (IL)-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays. Cytokine, **64**(3): 660–665. doi:10.1016/j.cyto.2013.09.018. PMID:24128872.

Villiger, P.M., Terkeltaub, R., and Lotz, M. 1992. Monocyte chemoattractant protein-1 (MCP-1) expression in human articular cartilage. Induction by peptide regulatory factors and differential effects of dexamethasone and retinoic acid. J. Clin. Invest. **90**(2): 488–496. doi:10.1172/JCI115885. PMID:1365641.

Wandel, S., Juni, P., Tendal, B., Nuesch, E., Villiger, P.M., Welton, N.J., et al. 2010. Effects of glucosamine, chondroitin, or placebo in patients with osteoarthritis of hip or knee: network meta-analysis. BMJ, **341**: c4675. doi:10.1136/bmj.c4675. PMID:20847017.

Wojdasiewicz, P., Poniatowski, L.A., and Szukiewicz, D. 2014. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediat. Inflamm. **2014**: 561459. doi:10.1155/2014/561459. PMID:24876674.

Zdzieblik, D., Oesser, S., Gollhofer, A., and Koenig, D. 2016. Collagen peptides reduce knee joint discomfort in physically active young adults. Int. J. Sports Exerc. Med. **2**: 041.

Zhang, W., Robertson, J., Jones, A.C., Dieppe, P.A., and Doherty, M. 2008. The placebo effect and its determinants in osteoarthritis: meta-analysis of randomised controlled trials. Ann. Rheum. Dis. **67**(12): 1716–1723. doi:10.1136/ard.2008.092015. PMID:18541604.