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Effects of specific collagen peptide supplementation combined with resistance training on Achilles tendon properties

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The purpose of this study was to investigate the effect of specific collagen peptides (SCP) combined with resistance training (RT) on changes in tendinous and muscular properties. In a randomized, placebo-controlled study, 40 healthy male volunteers (age: 26.3 ± 4.0 years) completed a 14 weeks high-load resistance training program. One group received a daily dosage of 5g SCP while the other group received 5g of a placebo (PLA) supplement. Changes in Achilles tendon cross-sectional area (CSA), tendon stiffness, muscular strength, and thickness of the plantar flexors were measured. The SCP supplementation led to a significantly ($p = 0.002$) greater increase in tendon CSA (+11.0%) compared with the PLA group (+4.7%). Moreover, the statistical analysis revealed a significantly ($p = 0.014$) greater increase in muscle thickness in the SCP group (+7.3%) compared with the PLA group (+2.7%). Finally, tendon stiffness and muscle strength increased in both groups, with no statistical difference between the groups. In conclusion, the current study shows that the supplementation of specific collagen peptides combined with RT is associated with a greater hypertrophy in tendinous and muscular structures than RT alone in young physically active men. These effects might play a role in reducing tendon stress (i.e., deposition of collagen in load-bearing structures) during daily activities.

KEYWORDS
cross-sectional area, protein supplementation, stiffness, tendon

1 | INTRODUCTION

Human tendons have various functions as a link between muscles and bones[1] and are essential for the execution of locomotor tasks and other movements.[2] In addition to transferring muscular forces to the skeletal system, the tendinous tissue is able to store and release energy and creates favorable conditions for optimizing muscle force production.[3,4] Considering the tight link between the muscular and tendinous apparatus, tendon adaptations, alongside with muscular adaptions, are indispensable for maintaining movement economy and reducing injuries.[2] High-load resistance training is efficient to augment the adaptive response of tendons by stimulating tenocytes to produce extracellular matrix (ECM) proteins.[1] As a consequence, tendon stiffness[5] and cross-sectional area[3] improve following high-load resistance training.

There is evidence, that these anabolic processes can be positively influenced by the administration of collagen peptides. In vitro experiments have demonstrated that collagen peptides can stimulate tenocytes to synthesize ECM molecules such as elastin and collagen type I and III.[6] The potential of collagen peptides to enhance the ECM protein synthesis might be responsible for the benefits of collagen-derived supplements in tendon architecture and functionality. Collagen peptides are characterized by a low molecular weight and high proportion of proline and hydroxyproline leading to high resistance to intestinal digestion and higher transport efficiency and consequently to a high bioavailability.[7–9]

A case report from a professional basketball player with Patellar Tendinopathy revealed a progressive decrease in MRI reactivity at the inferior pole of the patella after the daily intake of 15 g gelatin and 225 mg vitamin C in combination with a rehabilitation program for 18 months. As a potential consequence, pain symptoms and performance improved.[10] The results of a recent study with patients suffering from Achilles tendinopathy demonstrated that the intake of specific collagen peptides combined with an eccentric exercise program significantly reduced tendon lesions. In addition, an improvement in pain symptoms, function in daily living, and sporting activity as measured by Victorian Institute of Sports Assessment–Achilles (VISA-A) questionnaires were identified.[11] There is also evidence, that the administration of collagen peptides improves perceived function of the ankle in chronic ankle instability, which is closely related to impaired biomechanical properties of the Achilles tendon.[12] At the muscular level, recent studies showed an enhancing effect of exercise-associated collagen supplementation on muscle strength and fat-free mass, at least in populations of women and older men, respectively[13,14] In addition, a more pronounced increase in fat-free mass was demonstrated by resistance training with collagen supplementation in recreationally active men compared to a placebo group.[15] Although fat-free mass is not necessarily predictive of changes in muscle structure, previous in-vivo studies indicated significant upregulations of important markers of muscle protein synthesis (e.g., mTOR and p70S6k).[16] However, these results are in contrast to studies that found less-pronounced effects on muscle mass[17] compared to whey protein.

Whilst first clinical studies provide evidence that collagen peptides in combination with a training intervention enhances the rehabilitation of injured tendons, the beneficial effects on structural and mechanical properties of the healthy Achilles tendon remain unknown. In this context, the primary objective of the present randomized-controlled trial was to investigate the effects of 14 weeks of heavy resistance training with additional SCP supplementation on tendon CSA and stiffness (structural and mechanical properties). Furthermore, changes in maximal voluntary torque and muscle thickness of the gastrocnemius muscle were evaluated. Based on previous research, we hypothesize that training-associated supplementation with specific collagen peptides has an enhancing effect on mechanical and morphological adaptations of the Achilles tendon.

2 | METHODS

This was a double-blind, placebo-controlled, parallel-group study design with balanced randomization. Each participant was block randomized to one of the two groups before baseline measurements by a computer software. The person responsible for randomization was not involved in the data collection and analysis. All data were collected and analyzed at the Department of Sport and Sport Science of the University of Freiburg, Germany.

2.1 | Subjects

Forty healthy men aged between 18 and 40 years (Body Mass index =18.9–29.9 kg/m^2) with no prior lower body injuries who had no recent history (>12 months) of resistance training or who are not involved in any systematic physical training (>60 min per week) volunteered to participate. The participants were recruited by the announcement on social media, local newspapers, and flyers in different departments of the University of Freiburg. Cardiovascular diseases, contraindications to physical activity in accordance with American College of Sports medicine (ACSM) guidelines or contraindications to the intake of collagen peptides, or maltodextrin were defined as exclusion criteria.

The study interventions were approved by the Ethics Committee of the University of Freiburg (516/17-190080) and the trial was registered at the German Clinical Trials Register (DRKS00015998). All methods were performed in accordance with the relevant guidelines and regulations.

After being informed about the purpose and risks of the study, written informed consent was obtained and participants were randomly assigned to the SCP group or PLA group.

Prior to the intervention, subjects underwent a screening examination with medical anamnesis to check for accordance with the inclusion criteria. Any subject with an acute injury or lower extremity injury within the past year was excluded from participation. Before and after the 14 weeks intervention, all subjects passed through a testing procedure at the Department of Sport and Sport Science at the University of Freiburg, Germany. The data were not unblinded until data collection and database lock were both completed.

2.2 | Achilles tendon morphology

Changes in the structural tendon properties of the Achilles tendon were determined by measuring the cross-sectional area.

Transversal b-mode ultrasound images [8MHz, ArtUs EXT-1H, Telemed, Vilnius, Lithuania] were used to assess cross-sectional area at the distal 25% of the Achilles tendon length with a 6 cm wide transducer [LV 7.5/60/128Z-2; UAB Telemed,]. This location was chosen since at this location, the visibility of the Achilles tendon had the highest spatial resolution, and the boarders of the Achilles tendon did not exceed the transducer's field-of-view. The Achilles tendon length was defined as the distance from the tuberositas calcanei to the most proximal part of the musculotendinous junction toward the m. gastrocnemius medialis. Images were manually analyzed with ImageJ [1.51, NIH,] (Figure 1). Two independent, fully blinded assessors conducted three measurements for each image. The mean result of both assessors was used for further calculations. This was implemented to minimize intra-rater variability. In a group of ten subjects, who were reassessed after 72 h, the measurement showed an inter-day coefficient of variation (CV) of 4.8%.

2.3 | Achilles tendon function

Tendon mechanical properties were measured by calculating the Achilles tendon stiffness. For that purpose, the Achilles tendon elongation, changes in ankle angle, and ankle torque during isometric plantar flexions were recorded. The subjects were placed in a dynamometer [ISOMED 2000, Ferstl,] with their right ankle fixed to the dynamometer lever arm, which was set at 90° of plantar flexion. Subjects laid in prone position, with fixed shoulder and hip as well as fully extended knee and hip joints. The detailed protocol for the analysis of the elongation of the Achilles tendon during plantar flexion is described elsewhere[18]; therefore, a brief explanation of our approach is provided below.

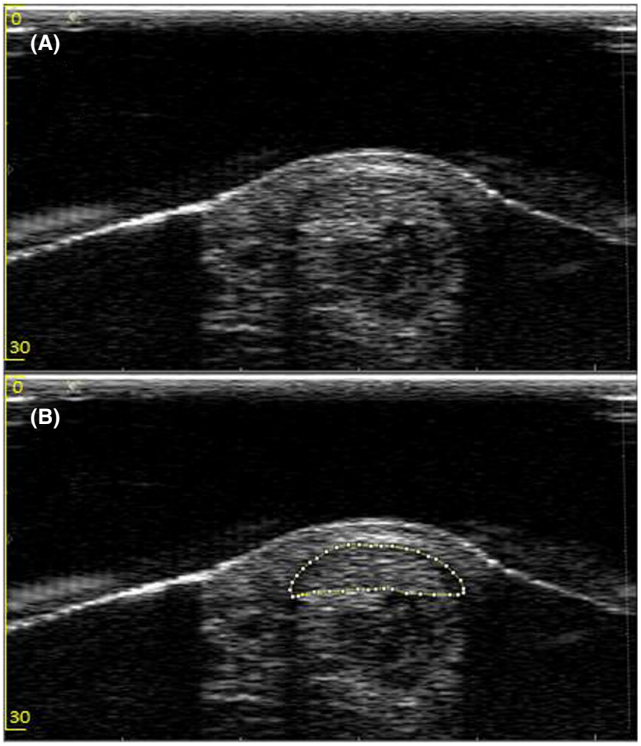

FIGURE 1 Ultrasound Scan of the Achilles tendon at 25% distal length (A). Manual analyzation of the CSA with ImageJ (B)

After preconditioning of the tendon consisting of five isometric contractions with 80% of the individual MVC,[19] the subjects were instructed to perform maximal isometric force ramps with a standardized loading rate of 50 Nm/s. With this loading rate, all subjects reached their maximal plantar flexion torque within 3–5 s. Subjects received visual feedback about their torque in order to ensure a constant loading rate. To ensure high reliability, five attempts were recorded for each subject.

During the contractions, movement of the junction between gastrocnemius medialis and AT was recorded by B-mode ultrasound scans at 100 Hz. Therefore, a handheld linear array ultrasound transducer was placed on the myotendinous junction so that a distal insertion of a fascicle into the epimysium was clearly visible. The displacement of the insertion during contraction was analyzed by a semi-automatic tracking software (Tracker, V 4.95) and determined as AT elongation. To detect potential probe movements, hypoechoic markers were placed on the skin and the ultrasound probe.

To correct the tendon elongation for ankle movement, 2D motion analyses of the ankle angle changes were conducted. Three LED markers were attached to the tibia, malleolus medialis, and metatarsal bone. Movement of the markers was recorded by a camera in sagittal plane and automatically tracked by 2D motion software [Simi Motion, Munich, Germany] (Figure 2).

Plantar flexion torque was recorded at 1000 Hz. It was divided by the Achilles tendon moment arm in order to calculate the Achilles tendon force. To assess the individual tendon moment arm, the perpendicular distance from the line of action of the Achilles tendon to the center of rotation of the ankle was measured by sagittal plane pictures and US (ultrasound) records. The exact method for measuring the Achilles tendon moment arm is described in detail elsewhere.[20] All graphical analysis was conducted with ImageJ [1.51, NIH,].

Data of Achilles tendon lengthening, ankle movement, and plantar flexion torque were filtered using a second-order low-pass butterworth filter (15 Hz cut-off frequency). Achilles tendon stiffness was defined as the mean slope of the force-elongation curve between 50%–80% of the individual MVC of the baseline measurement.

2.4 | Maximum voluntary plantar flexion torque

Furthermore, the isometric maximum voluntary plantar flexion torque was measured at 90° ankle angle using an isokinetic dynamometer [ISOMED 2000, Ferstl,]. All subjects were, therefore, placed in a prone position with extended knee and hip as described above. The highest of three MVCs was used for data analysis.

2.5 | Muscle thickness

There is a strong structural and functional relationship between the muscle and tendon. Muscle thickness of the right medial part of the gastrocnemius muscle was, therefore, also defined as secondary endpoint and assessed using B-mode ultrasonography [Aplio 400; Toshiba,]. Ultrasound-derived muscle thickness has repeatedly been found to be a useful and reliable marker of muscle growth following resistance training intervention.[21] Participants were lying in a prone position with their knee joints fully extended. The ankle position was fixed at 90° by the help of a custom-build orthosis.[20] After a resting period of 20 min, three sagittal images were obtained at 30% of tibia length (from popliteal crease to lateral malleolus)[20] using a 60 mm wide 9-MHz transducer. During all procedures, a thick ultrasound gel layer was used to minimize pressure on the underlying muscle tissue. In this context, each image was manually checked for an identifiable layer of ultrasound gel between the probe and the skin. For analyses, the shortest distance between the upper and lower aponeurosis was measured at 25%, 50%, and 75% of the image's field-of-view (Figure 3). Each of the three images was analyzed three times [ImageJ 1.51; NIH, Bethesda, MD] and the mean of all three measurement sites were used for statistical analysis.

The participants were instructed to maintain their habitual dietary intake and normal physical activity during the study. To control for potential alterations in nutrition, macronutrient status was tracked before and after the intervention. For that purpose, the subjects were advised to record their dietary intake on 3 days (2 weekdays, 1 weekend day). The nutritional logs were analyzed with Nutriguide 4.6 [Nutri Science GmBH,]. At the same time points, the subjects' status of physical activity was assessed by the Freiburg Questionnaire of physical activity.[22]

2.6 | Training

Three training sessions per week were conducted during the 14 weeks intervention period. To ensure proper recovery, two trainings were separated by a resting day. The training sessions started with a standardized warm-up of 10 min on a cycle ergometer (50–80 Watt).

In each training session, the subjects conducted two exercises with three sets of dynamic calf raises. One was conducted in sitting (Body-Solid Seated Calf Raise Machine, GSCR349,) and the other one in standing (Multipress, Genius Eco, FREI AG,) position. At the beginning and after every fourth week of the training period, the individual 1RM was tested in order to adjust the training weight to the actual strength level of each subject. In the same intervals, the load was progressively increased from 70% to 85% of the 1RM and the repetitions per set decreased from 12–6. For each exercise, time under tension during one repetition was 4s with resting periods between sets and exercises of 1 and 3 min, respectively. The participants were instructed to perform all exercises in full range of motion (full plantar

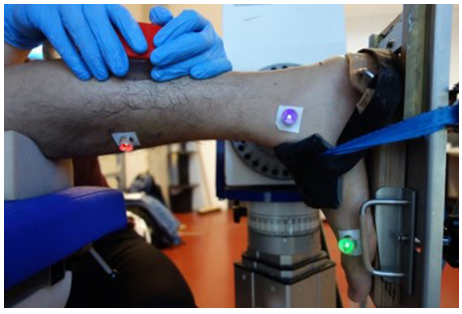

FIGURE 2 Image of measurement of the Achilles tendon stiffness

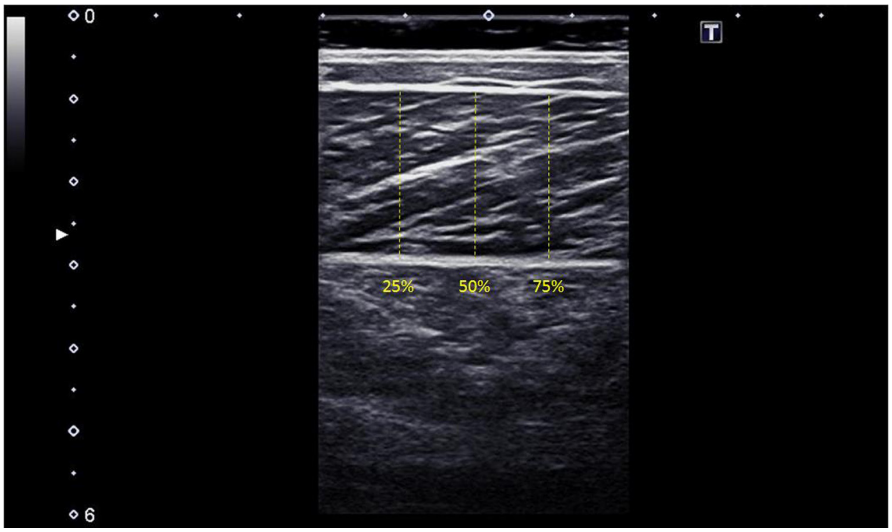

FIGURE 3 Ultrasound Scan of the medial part of the gastrocnemius muscle. Manual analyzation of the muscle thickness at 25%, 50%, and 75% of field-of-view with ImageJ

flexion to full dorsal flexion). To ensure correct execution, all trainings were monitored by experienced sport scientists. Additionally, to the calf raises, core stability exercises (front-planks; side-planks; bridging) were performed to increase the subjects' compliance with the training intervention.

2.7 | One repetition maximum assessment

Before the implementation of a new training load (at the beginning of weeks 1, 5, 9, and 13) dynamic 1RMs were assessed for both calf raising exercises to adjust the training load to the current strength level of each subject. An exercise-specific warm-up program of sets with 10 and 3–5 repetitions (two sets each) was conducted. Afterward, the maximal weight that a subject was able to lift through full range of motion was assessed. Therefore, the load was increased by 5%–10% after each successful repetition until the subjects failed to lift it into full dorsal flexion or lifted with poor technique.[23] To assure full recovery, there was a four-minute rest between each 1RM attempt.

2.8 | Supplementation

Participants in the SCP group received a daily dose of 5 g of a specific mixture of specific collagen peptides (TENDOFORTE, GELITA AG,). For amino acid composition see Table 1. The same dose of maltodextrin was supplemented in the PLA group. Both water-soluble preparations could not be distinguished by taste or optical appearance. The participants ingested the supplement dissolved in ~250ml water within one hour after each training session and at the same daytime on non-training days.

TABLE 1 Amino acid composition of the collagen peptides

Amino acid	Weight (%)	Mol (%)
Hydroxyproline	11.3	9.6
Aspartic acid	5.8	4.8
Serine	3.2	3.4
Glutamic acid	10.1	7.5
Glycine	22.1	32.3
Histidine	1.2	0.8
Arginine	7.8	5.0
Threonine	1.8	1.7
Alanine	8.5	10.5
Proline	12.3	11.8
Tyrosine	0.9	0.5
Hydroxylysine	1.7	1.2
Valine	2.4	2.3
Methionine	0.9	0.9
Lysine	3.8	2.9
Isoleucine	1.3	1.1
Leucine	2.7	2.3
Phenylalanine	2.1	1.4

2.9 | Statistics

The statistical software package SPSS version 24.0 [IBM,] was used for all statistical analysis. After confirming normal distribution and variance homogeneity, a repeated-measures ANOVA (rmANOVA) was calculated in order to examine potential time and interaction (time ×group) effects for all variables. Furthermore, paired *t*-tests within groups were conducted. For all analyses, outliers detected via Grubb's test were removed.[24]

All following data are presented as mean ± SD. Additionally, the partial eta-squared was calculated as a measure of effect size (small effect: η_{p^2} >0.01, medium effect: η_{p^2} >0.06, large effect: η_{p^2} >0.14). The alpha level was set to $p < 0.05$.

3 | RESULTS

A total of $n = 27$ participants completed the 14 weeks intervention period, with 6 and 7 dropouts in the PLA and SCP group, respectively. All dropouts were related to noncompliance with the study protocol (or loss to follow-up). None of the dropouts were associated with any side effects or adverse events caused by the training program or the intake of the investigational products. Baseline characteristics of the analyzed participants are shown in Table 2. There were no statistically significant differences between the groups in any of the anthropometric variables or outcome criteria.

3.1 | Achilles tendon cross-sectional area

Following 14 weeks of resistance training, tendon CSA increased from 67.8 ± 10.9 mm² to 75.2 ± 12.4 mm² in the SCP group and from 75.4 ± 16.3 mm² to 78.5 ± 16.5 mm² in the PLA group (Figure 4A). As indicated by the results of the paired sample t-test ($p < 0.001$) and the main time effect of time ($p < 0.001$, η_{p^2} =0.733) in the rmANOVA, significant improvements in Achilles tendon cross-sectional area were detected in both groups. The significant time ×group interaction ($p = 0.002$, η_{p^2} =0.312) showed, that the specific collagen peptide supplementation led to a statistically relevant greater gain in Achilles tendon cross-sectional area than placebo.

TABLE 2 Anthropometric characteristics ($n = 27$)

Variable	Group	(M ± SD)
Age (years)	PLA	26.1 ± 4.2
	SCP	26.5 ± 3.9
Height (cm)	PLA	179.7 ± 9.2
	SCP	180.8 ± 8.9
Weight (kg)	PLA	76.4 ± 15.4
	SCP	77.9 ± 12.1
BMI (kg/m²)	PLA	23.5 ± 3.5
	SCP	23.7 ± 2.8

Abbreviations: M, mean; PLA, Placebo; SD, standard deviation; SCP, Specific Collagen Peptides.

3.2 | Achilles tendon stiffness

The rmANOVA demonstrated a statistically significant main effect of time ($p < 0.01$ η_{p^2} =0.346) but no interaction effect ($p = 0.192$, η_{p^2} =0.073). On average, tendon stiffness in the SCP group increased from 389.5 ± 135.0 N/mm to 453.7 ± 158.8 N/mm and in the PLA group from 401.5 ± 102.6 N/mm to 541.1 ± 132.5 N/mm (Figure 4B). Paired sample t-test (SCP: $p < 0.05$; PLA: $p < 0.01$) showed significant increases in both groups.

3.3 | Muscle thickness

Muscle thickness of the gastrocnemius muscle changed from 2.18 ± 0.24 cm to 2.34 ± 0.22 cm and from 2.15 ± 0.42 cm to 2.20 ± 0.39 cm in the SCP and PLA groups, respectively (Figure 4D). As indicated by the results of the paired sample t-test ($p < 0.001$) and the main effect of time ($p < 0.001$; η_{p^2} =0.559) in the rmANOVA, significant improvements in muscle thickness were detected in both groups. The significant time ×group interaction ($p = 0.014$; η_{p^2} =0.218) revealed in the SCP group a statistically relevant greater gain in muscle thickness than in the PLA group. Paired sample t-test (SCP: $p < 0.001$; PLA: $p < 0.01$) showed significant increases in both groups.

3.4 | Maximum voluntary torque

After 14-weeks of resistance training and supplementation, both groups showed improved levels of maximum voluntary torque (time effect: $p < 0.001$; η_{p^2} =0.587). No statistically significant interaction effect was observed ($p = 0.629$; η_{p^2} =0.009). In this context, maximal voluntary torque in the SCP group increased from 212.8 ± 58.4 Nm to 234.5 ± 64.5 Nm and in the PLA group from 189.0 ± 83.1 Nm to 214.6 ± 86.1 Nm (Figure 4C). Paired sample t-test (SCP: $p < 0.01$; PLA: $p < 0.001$) showed significant increases in both groups.

3.5 | Dietary intake and physical activity

The two groups did not differ significantly in nutritional intake or physical activity at the baseline testing. Furthermore, rmANOVA showed no significant time-group interaction regarding protein ($p = 0.583$, η_{p^2} =0.012), fat ($p = 0.631$, η_{p^2} =0.009) and carbohydrate ($p = 0.540$, η_{p^2} =0.015) consumption (Table 3) as well as total energy intake ($p = 0.738$, η_{p^2} =0.005) or weekly hours spent with physical activity ($p = 0.510$, η_{p^2} =0.018).

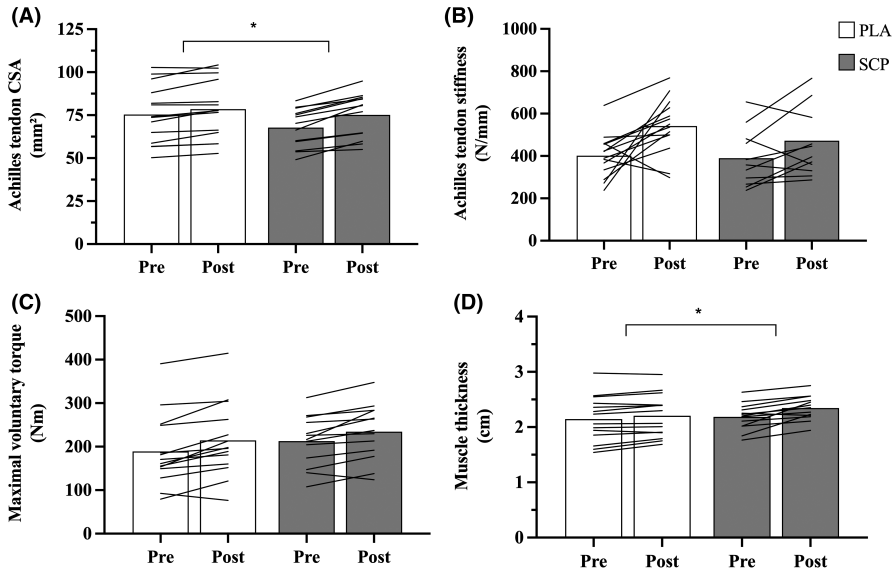

FIGURE 4 Mean values of tendon cross-sectional area (A), Achilles tendon stiffness (B), Maximal voluntary torque (C) and muscle thickness of the gastrocnemius muscle (D) before (pre) and after (post) the 14-week resistance training intervention. White rectangles =placebo group, gray rectangles =specific collagen peptide group. Straight lines represent individual cases. * indicates a significant time ×group interaction ($p < 0.05$) in favor of the specific collagen peptide group

4 | DISCUSSION

The main purpose of the current trial was to investigate the effect of the daily intake of specific collagen peptides in combination with a high-load lower-body resistance training on changes in Achilles tendon CSA and stiffness and, in addition, on changes in maximal voluntary torque and muscle thickness.

To our best knowledge, this is the first study that investigated the effects of a collagen peptide supplementation on training-induced adaptions of mechanical and structural tendon properties in healthy participants in vivo.

Consuming 5 g of specific collagen peptides per day for 14 weeks combined with resistance training for the lower extremities led to a statistically significant increase in Achilles tendon CSA compared with resistance training only. Furthermore, thickness of the gastrocnemius muscle increased statistically significantly greater in the group receiving specific collagen peptides.

An increase in Achilles tendon CSA after resistance training was confirmed in the present study. The 14-weeks of resistance training led to a statistically significant effect in both groups ($p < 0.001$). Furthermore, the results show a significantly more pronounced CSA increase in the SCP group compared to the PLA group ($p < 0.01$).

With a tendon CSA increase of 4.7%, the results of the PLA group are in accordance with previous research in which comparable interventions with resistance training reported adaptations between 0.5 and 12.9%.[25] Interestingly, the group receiving a daily dosage of SCP increased their tendon CSA by 11.0%, which was significantly higher compared with the PLA group.

Tendon hypertrophy following training in general and training with concomitant supplementation, in particular, is for now largely unexplained by the knowledge from previous research. Several clinical observational studies showed a stimulating effect of resistance training on the cross-sectional area of the Achilles tendon.[2,25] So far, there is only one clinical study showing that 12 weeks of resistance training and concomitant protein supplementation has a promoting impact on patellar tendon CSA adaptions.[26] The extent to which an increased collagen synthesis leads to an increased tendon CSA could not be fully clarified. Within a training intervention, Kubo et al. (2012) demonstrated an increase of procollagen type I C-peptide in blood samples and deduced a rising collagen content from an increase in echo intensity of the tendon.[5] In contrast, no alteration of the collagen biosynthesis was observed in tendon biopsies by Heinemeier et al. (2013) and Eriksen et al.[27,28] Further investigations demonstrate that a (training-induced) increase of tendon CSA is not accompanied by increased collagen synthesis in the core region of the tendon.[29]

However, these studies did not include any additional protein supplementation. In a placebo-controlled study by Shaw et al., (2017), healthy male subjects who supplemented vitamin C-enriched collagen supplements before

TABLE 3 Dietary assessment of macronutrients at pre ($n = 27$)

Variable	Group	($M \pm SD$)
Protein (g/day)	PLA	93.6 ± 62.6
	SCP	87.1 ± 30.7
Fat (g/day)	PLA	93.2 ± 38.8
	SCP	82.7 ± 32.3
Carbohydrate (g/day)	PLA	254.4 ± 88.8
	SCP	248.6 ± 89.0

Abbreviations: M, mean; PLA, Placebo; SD, standard deviation; SCP, Specific Collagen Peptides; g, grams.

exercising showed increased blood concentrations of glycine, proline, hydroxyproline and hydroxylysine as well as amino-terminal propeptide of collagen I. Furthermore, engineered ligaments treated with the blood serum showed increased collagen content and the authors concluded that supplementation with gelatin augments collagen synthesis after exercise.[30] Additionally, results from a preclinical study on primary fibroblasts derived from human tendons and ligaments revealed a pronounced stimulatory effect on RNA expression and biosynthesis of collagen and other matrix molecules after adding specific collagen peptides.[6] These findings were supported by an in-vivo animal study by Minaguchi et al. (2005), in which rabbits that were fed with collagen peptides over a time period of 56 days showed increased collagen fibril diameter.[31] From a mechanistic point of view, the present changes in tendon CSA in the SCP group might, therefore, be explained by stimulatory effects of SCP on tendon matrix molecules, although the extent of collagen deposition within the mature tendon is currently under discussion in the scientific community.[27] As the present data do not allow to draw conclusions about collagen deposition, additional experiments are needed to provide a detailed mechanistic description of the nature of the tendon hypertrophy.

The significant changes in Achilles tendon stiffness can be explained by the resistance training. Both groups equally increased tendon stiffness throughout the 14 weeks resistance training intervention. With a relative increase of 34.7% in the total study population, the results are in accordance with previous findings. For instance, studies with 14 weeks of high-load isometric resistance training reported increases in stiffness between 24.8% and 53.9%, respectively.[3] Furthermore, in a study by Carroll et al.,[32] 12 weeks of dynamic high-load resistance training led to adaptions of 13.9%. In the present trial the two groups revealed no statistically significant difference in stiffness adaptions.

The observation of tendon hypertrophy of the SCP group without a proportionate increase in tendon stiffness might be explained by either deposition of non-load-bearing material in the SCP group, alterations of the tendon material properties by the SCP supplementation or by methodological factors.

It has to be mentioned that the magnitude of changes of CSA and tendon stiffness following resistance training indicates a variable individual response.[2] Furthermore, there is first evidence that the differences in Achilles tendon CSA are not reflected by differences in tendon stiffness[33] despite a close functional and anatomical connection.

A greater tendon CSA is accompanied by lower tendon stress at given tendon force levels. Thus, on a functional level, it may decrease the risk of overuse injuries[34] and could potentially play a role at compensation of imbalanced adaptions of muscle and tendon after resistance training.[35] However, functional consequences of stiffness adaptions depend on the type of exercise and cannot be drawn universally. For example, lower tendon stiffness is associated with better performances in long distance runs.[33] Additional investigations would be appropriate to establish whether a decrease in Young's modulus occurs as a result of collagen supplementation and to estimate the functional consequence of such an outcome.

The effects on ECM of the Achilles tendon might have an influence on the surrounding muscle tissue as well. There is evidence that the intramuscular ECM regulates the formation, maintenance and differentiation of myosatellite cells.[36] In the present study, muscle thickness increased in the SCP group to a statistically significantly greater extent (7.3%) than in the PLA group (2.7%).

The results of clinical trials identified an increased fat-free mass after the daily intake of 15 g specific collagen peptides with a mean molecular weight of 3.5 kDa over 12 weeks.[13,14,37] On a cellular level, however, the findings did not reveal significant changes in fiber type cross-sectional area.[38] According to the current state of research, the positive effect of collagen peptide supplementation on muscle hypertrophy may be due to the enhanced phosphorylation of muscle protein synthesis markers (e.g., Akt, mTOR, p70S6K)[16] and upregulated proteins of the contractile elements.[15]

In both groups, resistance training led to a significant increase in maximal voluntary isometric torque of the plantar flexions. However, no significant differences between the groups were observed. Previous studies have shown strong population-specific improvements in muscle strength. In women, Jendricke et al.[14] have shown that a SCP administration during 12 weeks of resistance training led to a more pronounced increase in leg muscle strength compared to a placebo control group. Similar effects have been confirmed by Oertzen-Hagemann et al. (2019) who investigated the effects of resistance training and SCP in young men.[15] In an untrained male study population, the improvements in leg strength were more pronounced in the training group receiving additionally SCP compared with the single resistance training regime.[37] In older individuals who already had compromised levels of muscle strength (e.g., sarcopenic individuals), evidence suggests that a simultaneous ingestion of SCP during resistance training can induce favorable adaptations on a statistically significant level.[13]

Interestingly, the hypertrophic response, which was seen in the SCP group was not accompanied by significantly different changes in maximal voluntary torque between the two groups. It is known from earlier studies that training-induced muscle hypertrophy does not necessarily

facilitate adaptations in strength tasks.[39] Not only muscle hypertrophy, but also neurophysiological, morphological adaptations and individual motivation have to be taken into account for changes in muscle strength.[40] These factors might be responsible for the lack of group differences in gains in maximal voluntary isometric torque, despite the significant higher hypertrophy effect in the SCP group.

These non-linear responses might also be explained by the principle of exercise specificity.[41] Since the training program consisted of dynamic and the testing of isometric exercise modes, it might be plausible that the transferability from training to testing was not sufficient.[41]

Previous data demonstrated that various collagen peptides differ in their pharmacological effects and thus in their efficacy due to different biochemical properties.[42] Consequently, the results of the present study cannot be extrapolated to other collagen peptide ingredients. Further studies are needed to confirm these findings in a clinical setting and investigate the potential mechanisms underlying the observed adaptations.

4.1 | Limitations

One limitation of the study was that tendon morphology was measured with ultrasonography on 25% of patellar tendon length. Conducted correctly, ultrasonography was found a suitable alternative for structural measurements of the Achilles tendon.[43–45] It, therefore, represents a frequently used method.[46–48] The location at which the tendon was measured was deliberately chosen to ensure accurate analysis of the tendon in an area where cross-sectional adjustments are expected. Nevertheless, the chosen method does not allow conclusions to be drawn about other areas of the tendon and, therefore, part of the adaptation may be overlooked.

The same limitation is reflected in the method used to determine muscle morphology. Due to the limited field-of-view of the ultrasound device and the restrictions in the measurement procedure, only the thickness of the medial portion of the gastrocnemius muscle could be assessed. As a result, hypertrophic adaptations in other areas of the triceps surae muscle cannot be elicited.

Despite a dropout rate of 32.5% post hoc calculations revealed sufficient study power for all tendon variables (power >0.9) indicating that no important effects were missed.

5 | PERSPECTIVE

The findings of the present study demonstrated that the intake of 5 g specific collagen peptides in combination with resistance training for 14 weeks leads to increased adaptions of structural properties of the Achilles tendon compared to a placebo group with the same training program.

The results of the primary endpoint of the study shows a statistically significant increase in tendon cross-sectional area following the ingestion of specific collagen peptides compared with a placebo. Furthermore, the supplementation of specific collagen peptides led to a statistically significant increase in muscle thickness. Muscle strength and tendon stiffness improved in both groups similarly. Although the underlying mechanisms have to be further elucidated, the current findings could be relevant for patients suffering from tendon overuse, such as tendinopathies, since increased tendon CSA leads to a concomitant decrease in tendon stress and thus the oral administration of specific collagen peptides might be beneficial for both prevention as well as rehabilitation of tendon injuries.

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CONFLICTS OF INTERESTS

S.O. is coinventor of patents concerning the use of collagen peptides. The other authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

SJ designed research, performed experiments, analyzed data, interpreted results of experiments, prepared figures, and drafted and edited the manuscript. CC designed research, performed experiments, analyzed data, interpreted results of experiments, prepared figures, drafted and revised manuscript. BL designed research, analyzed data, interpreted results of experiments, edited and revised manuscript. ORS designed research, interpreted results of experiments, edited and revised manuscript. TS designed research, performed experiments, analyzed data, interpreted results of experiments. PJ designed research, edited and revised manuscript. SO designed research, edited and revised manuscript. AG designed research, edited and revised manuscript. DK designed research, interpreted results of experiments, edited and revised manuscript. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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