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Impact of long-term collagen peptide supplementation in combination with physical training on recovery following exercise-induced muscle damage, body composition and musculotendinous adaptations

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Abstract

Sports nutrition has become increasingly vital as the connection between diet and athletic performance is now better understood, with advancements in research providing precise nutritional strategies that enhance performance, recovery, and overall health, and despite collagen peptides being viewed as a low-quality protein, they have gained significant scientific interest for their potential benefits in sports performance and injury prevention. Collagen peptides are typically derived from animal sources like porcine, bovine, chicken, and marine, followed by extraction methods such as chemical or enzymatic hydrolysis. Collagen peptide absorption and effectiveness are influenced by their molecular weight, with smaller peptides, particularly di- and tripeptides, being more readily absorbed through mechanisms like PEPT1 transport. Evidence from exercise trials shows that eccentric contractions significantly boost collagen and growth factor expression in tendons and muscle tissues, and that collagen synthesis peaks 24-48 hours post-exercise, coordinated with myofibrillar protein synthesis but at a slower rate. Hormonal differences, particularly estrogen levels, influence collagen synthesis, with studies showing that females may have lower collagen synthesis rates compared to males and that the menstrual cycle or hormonal contraceptives can further affect these rates. Combining collagen supplementation with resistance training enhances protein synthesis, though evidence shows that while whey protein often leads to greater myofibrillar protein synthesis, collagen supplementation may be more effective for collagen-rich tissues like tendons, particularly when higher doses are used (15 - 30 g). Research on the molecular effects of collagen peptide administration has demonstrated that collagen-specific dipeptides such as hydroxyprolyl-glycine (Hyp-Gly) and prolyl-hydroxyproline (Pro-Hyp) can activate key signaling pathways involved in muscle and tendon cell differentiation, growth, and repair, with some studies indicating that collagen peptides may enhance muscle and tendon regeneration in-vitro through pathways such as PI3K/Akt/mTOR and TGFβ-1/Smad.

Due to a lack of long-term trials investigating collagen peptide supplementation combined with regular exercise, papers 1 and 2 (out of three published as part of the PhD project) included a twelve-week concurrent training accompanied with 15 g of collagen peptide daily intake in low to moderately active males. Recovery was monitored through initial muscle damage-inducing exercise containing 150 drop jumps, followed by measurements before, 2h, 24h and 48h post-exercise. After obtaining isokinetic dynamometer and force plate related data (maximal voluntary contractions, rate of force development and countermovement jump height) as well as blood derived markers (creatine kinase, lactate dehydrogenase, myoglobin and C-reactive protein) and performing statistical analysis (mainly ANOVA with repeated measurements), results revealed significantly enhanced maximal, explosive,

and reactive strength compared to the placebo group. Moreover, diminished immediate muscle stress reaction and enhanced early-stage recovery was evident without inflammatory changes.

Paper 3 depicts a systematic review and meta-analysis revealing for the first time that prolonged collagen peptide ingestion (≥ 8 weeks) accompanied with physical training provokes increases in lean body mass, muscle mass, maximal strength and favorable adaptations in tendon morphology. Including short-term trials due to availability, recovery in reactive strength after exercise-induced muscle damage was also enhanced compared to a placebo. However, these findings are based on low to moderate certainty of evidence.

Overall, collagen peptide supplementation appears to significantly enhance the repeated bout effect by reducing muscle damage after repeated unaccustomed exercise, potentially through extracellular matrix remodeling, neural and musculotendinous adaptations. It may also improve tendon morphology and thus prevent tendon injuries. Additionally, collagen peptides could be a valuable dietary supplement for older adults, helping to maintain muscle mass, strength, and overall health, particularly when higher protein intake seems rather challenging.

Findings of this thesis suggest that collagen peptide supplementation may enhance collagen synthesis in mechanosensitive cells and tissues, possibly improving tissue response to mechanical stress through interactions with specific transmembrane receptors, the integrin family. This interaction could influence tissue mechanical properties, particularly in the context of exercise and tissue repair. The optimal dosage and amino acid composition of collagen supplements remain uncertain, highlighting the need for future studies to explore effective supplementation strategies, including combining collagen with other limiting amino acids or protein sources to maximize physiological benefits.

Abstract (Deutsch)

Die Sporternährung hat in den letzten Jahren zusehends an Bedeutung gewonnen, da der Zusammenhang zwischen Ernährung und sportlicher Leistung immer besser verstanden wird. Fortschritte in der Forschung offerieren präzise Ernährungsstrategien, die Leistung, Erholung und die allgemeine Gesundheit verbessern. Obwohl Kollagenpeptide oft als minderwertiges Protein betrachtet werden, haben sie aufgrund ihres Potenzials für sportliche Leistungssteigerung und Verletzungsprävention erhebliches wissenschaftliches Interesse geweckt. Kollagenpeptide werden typischerweise aus tierischen Quellen wie Schwein, Rind, Huhn und Meerestieren gewonnen, gefolgt von Extraktion mittles chemischer oder enzymatischer Hydrolyse. Die Absorption und Wirksamkeit von Kollagenpeptiden wird durch ihr Molekulargewicht beeinflusst, wobei kleinere Peptide, insbesondere Di- und Tripeptide, besser durch Mechanismen wie den PEPT1-Transport absorbiert werden. Studien mit Trainingsinterventionen zeigen, dass exzentrische Kontraktionen die Expression von Kollagen und Wachstumsfaktoren in Sehnen- und Muskelgeweben signifikant steigern und dass die Kollagensynthese 24 bis 48 Stunden nach dem Training ihren Höhepunkt erreicht. Diese erfolgt analog zur myofibrillären Proteinsynthese, verläuft jedoch langsamer. Hormonelle Unterschiede, insbesondere Östrogenspiegel, beeinflussen die Kollagensynthese, wobei Studien zeigen, dass Frauen möglicherweise niedrigere Kollagensyntheseraten haben als Männer und dass der Menstruationszyklus oder hormonelle Verhütungsmittel diese Raten weiter beeinflussen können. Die Kombination von Kollagen-Supplementierung mit Krafttraining erhöht die Proteinsynthese, obwohl Studien zeigen, dass während Molkenprotein häufig zu einer größeren myofibrillären Proteinsynthese führt, die Kollagensupplementierung für kollagenreiche Gewebe wie Sehnen, insbesondere bei höheren Dosen (15-30 g), wirksamer sein könnte. Forschungen zu den molekularen Effekten der Verabreichung von Kollagenpeptiden haben gezeigt, dass kollagenspezifische Dipeptide wie Hydroxyprolyl-Glycin (Hyp-Gly) und Prolyl-Hydroxyprolin (Pro-Hyp) bedeutende Signalwege aktivieren können, die an der Differenzierung, dem Wachstum und der Reparatur von Muskel- und Sehnengewebe beteiligt sind. Einige Studien deuten darauf hin, dass Kollagenpeptide die Muskel- und Sehnenregeneration in vitro über Signalwege wie PI3K/Akt/mTOR und TGFβ-1/Smad verbessern könnten.

Aufgrund eines Mangels an Langzeitstudien, die die Kollagenpeptidsupplementierung in Kombination mit regelmäßigem Training untersuchen, beinhalteten Paper 1 und 2 (von insgesamt drei, die im Rahmen des PhD-Projekts veröffentlicht wurden) eine zwölfwöchige Trainingsintervention, begleitet von einer täglichen Einnahme von 15 g Kollagenpeptiden bei wenig bis mäßig körperlich aktiven Männern. Die Regeneration wurde durch eine anfängliche, muskelkaterinduzierende Übung mit 150 Sprüngen aus einer Höhe von 60 cm

gemessen, gefolgt von weiteren Messungen unmittelbar vor, 2 Stunden, 24 Stunden und 48 Stunden nach den Sprüngen. Nach der statistischen Analyse (vorwiegend ANOVA mit wiederholten Messungen) von isokinetischen Dynamometer, Kraftmessplatte (maximale Kontraktionen, Kraftentwicklungsrate und Sprunghöhe) und Blut (Kreatinkinase, Laktatdehydrogenase, Myoglobin und C-reaktives Protein) stammenden Daten zeigten die Ergebnisse signifikant verbesserte Maximal-, Explosiv- und Reaktivkraft im Vergleich zur Placebogruppe. Darüber hinaus waren eine verringerte akute Muskelstressreaktion und eine verbesserte Erholung in der frühen Regenerationsphase ohne inflammatorische Änderungen zwischen den Gruppen erkennbar.

Die dritte Arbeit stellt eine systematische Übersichtsarbeit und Metaanalyse dar, die erstmals zeigt, dass die langfristige Einnahme von Kollagenpeptiden (≥ 8 Wochen) in Verbindung mit körperlichem Training zu einer Zunahme der fettfreien Körpermasse, der Muskelmasse, der Maximalkraft und zu günstigen Anpassungen in der Sehnenmorphologie führt. Auch in Studien mit kurzer Dauer, die aufgrund der geringen Verfügbarkeit einbezogen wurden, zeigte sich eine verbesserte Regeneration der Reaktivkraft nach muskelkaterinduzierenden Übungen im Vergleich zu einem Placebo. Diese Ergebnisse basieren jedoch auf einer geringen bis mittleren Evidenzvertraulichkeit.

Insgesamt scheint die Kollagenpeptidsupplementierung den Repeated-bout Effekt erheblich zu verbessern, indem sie Muskelschäden bzw. -kater nach wiederholten ungewohnten Übungen reduziert, möglicherweise durch Umbau der Extrazellulärmatrix, neuronale und muskulotendinöse Anpassungen. Es könnte auch die Sehnenmorphologie verbessert werden und so Sehnenverletzungen vorbeugen. Zusätzlich könnten Kollagenpeptide ein wertvolles Nahrungsergänzungsmittel für ältere Erwachsene sein, um Muskelmasse, Kraft und allgemeine Gesundheit zu erhalten, insbesondere wenn eine hohe Proteinzufuhr eine große Hürde darstellt.

Die Ergebnisse dieser Arbeit legen nahe, dass die Kollagenpeptidsupplementierung die Kollagensynthese in mechanosensitiven Zellen und Geweben verbessern und möglicherweise die Gewebereaktion auf mechanischen Stress durch Interaktionen mit spezifischen Transmembranrezeptoren, der Integrinfamilie, verbessern könnte. Diese Interaktion könnte die mechanischen Eigenschaften von Geweben beeinflussen, insbesondere im Kontext von Bewegung und Gewebsreparatur. Die optimale Dosierung und Aminosäurezusammensetzung von Kollagenpräparaten bleibt ungewiss, was den Bedarf an zukünftigen Studien unterstreicht, die wirksame Supplementierungsstrategien erforschen, einschließlich der Kombination von Kollagen mit anderen limitierenden Aminosäuren oder Proteinquellen, um physiologische Vorteile zu maximieren.

1. Introduction

Sports nutrition has become increasingly important in today's world as the link between diet and athletic performance is more clearly understood. With advancements in scientific research, elite athletes, fitness enthusiasts and recreationally active individuals now have access to precise nutritional strategies that can enhance training, improve performance, and expedite recovery. Sports nutrition is not just about improving athletic prowess, but also about maintaining overall health and preventing injuries, making it a critical component of any athlete's regimen. As evidenced by a simple search in PubMed using “protein supplementation” leading to 37,785 results since 2014, protein as an ergogenic aid depicts one of the most investigated nutritional supplements so far. According to a market analysis report (1), the global market size of protein supplements reached 5.8 billion US dollars in 2022 and is expected to grow at an 8% compound annual growth rate to 2030, highlighting also their tremendous economic impact. Collagen peptides as one of few animal-derived protein fractions have perpetually gained scientific interest in the last two decades not only for aesthetic purposes but also as a beneficial adjunct for sports performance and injury prevention. Compared to other proteins obtained from mammalian sources such as whey or casein, collagen is often portrayed as low quality protein due to its relatively low content of essential amino acids (EAA) that is typically assessed by the Protein Digestibility Corrected Amino Acid Score (PDCAAS) or the Digestible Indispensable Amino Acid Score (DIAAS) (2). A number of studies have already reported that collagen peptides may help alleviate joint pain in osteoarthritis patients, reduce movement-specific pain and discomfort in athletes, increase chondroprotection and bone mineral density, and potentially stimulate bone growth (3–7). In addition, collagen peptides have been shown to potentially improve body composition by increasing lean body mass, enhancing muscular strength and recovery, and stimulating muscle and tendon adaptations with injury-preventive benefits (8–10). To gain a deeper understanding of these potential effects, the following chapters will elucidate the microscopic structure, function and biosynthesis of collagen, the industrial production of collagen peptides, uptake patterns following ingestion, exercise and collagen peptide intake-induced de novo synthesis and involved molecular mechanisms leading to aforementioned adaptations. Afterward, the rationale and hypotheses of the PhD project (including three published papers, see Section 2) will be presented, followed by the three published full-text articles, methodological considerations, a discussion and an outlook on future research.

1.1 Molecular structure of collagen

The term "collagen" encompasses an entire family (of glycoproteins) distinguished by the following key features (11):

- The amino acid repeating sequence $[Gly–X–Y]_n$, which can occur with or without interruptions, with glycine (Gly) being the most abundant amino acid.
- The presence of proline and hydroxyproline in the X and Y positions, respectively.
- The formation of a right-handed triple helix from three left-handed polyproline α-chains of the same length, resulting in collagen's unique quaternary structure.

Various types of collagen possess unique amino acid compositions and perform specific functions in the body. A brief overview of the currently known 28 types of collagen is demonstrated in **Table 1**. Controversy exists to whether type 29 is classified as a new type due to its associated COL29A1 gene was reported to be identical to the COL6A5 gene (12). Depending on their supramolecular assembly, collagens can be divided into fibril-forming, fibril-associated, network-forming, beaded and anchoring filaments, transmembrane collagens and multiplexins. Fibrillar collagens are characterized by a single, prominent triple-helical domain. In contrast, collagens in the multiplexins category feature multiple triple-helical domains interspersed with unique non-triple-helical regions, which likely provide molecular flexibility. Transmembrane collagens serve dual roles, functioning as both cell surface receptors and matrix molecules. They facilitate interactions between the epithelium and mesenchyme during morphogenesis, aid in cell adhesion within epithelial tissues and nerves. Fibril-associated collagens do not form fibrils on their own but instead bind to the surface of collagen fibrils, helping to stabilize and maintain their integrity. Network-forming collagens generate networks, which are critical structural components of basement membranes, aiding in molecular integration. Beaded collagens intertwine with other collagen fibrils and are distinguished by their unique beaded filament structure (13). The physiological significance of collagen is particularly evident in genetic disorders like Ehlers–Danlos syndromes, which result from mutations in collagen genes COL1A1, COL1A2, COL3A1, COL5A1, COL5A2 and COL5A3. The Ehlers–Danlos syndromes encompass a diverse group of diseases marked by the fragility of soft connective tissues and manifesting broadly in the skin, ligaments, joints, blood vessels, and internal organs causing hyperlaxity, vascular complications, kyphoscoliosis and dermatosparaxis (skin fragility) (14). This highlights the essential and manifold roles of collagen proteins in the human body.

Table 1: Collagen type, group, location and function according to Mavropalias et al. (2023) (13). FACIT = Fibril associated collagens with interrupted triple helices, Multiplexin = Multiple triple-helix domains and interruptions, ECM = Extracellular matrix.

Type	Group	Location/Function
I	Fibril-forming	Comprises 90% of all mammalian collagen; skin, tendon, bone, ligaments, and muscular connective tissues
II	Fibril-forming	Cartilage
III	Fibril-forming	Forms a looser meshwork of fibers compared to collagen I, providing greater compliance to the tissue
IV	Network-forming	Basement membranes; forms networks, builds a scaffold that integrates ECM molecules
V	Fibril-forming	Particularly in the cornea; regulates initiation of collagen fibril assembly
VI	Beaded filament	Ubiquitous; cytoprotective, autophagy regulation, cell differentiation and apoptosis, oxidative damage protective
VII	Anchoring	Particularly in the skin; binding collagen I and III, thus providing ECM stability
VIII	Network-forming	Synthesized by endothelial cells: Descemet's membrane, brain, lung, liver, heart, and muscles, around chondrocytes; endothelial organization, angiogenesis
IX	FACIT	connected to the surface of collagen II fibrils in cartilage
X	Network-forming	Cartilage; distribution of proteoglycans and matrix vesicles within the growth plate
XI	Fibril-forming	Cartilage; controlling diameter and spacing of collagen II fibrils
XII	FACIT	Muscle; Interacts with ECM proteins, might stabilize the structure of collagen fibrils during development and remodeling
XIII	Transmembrane	Functions in the myotendinous junctions of muscles, assists in muscle fiber and basement membrane integrity
XIV	FACIT	Tendon, cornea, skin, and articular cartilage; involved in fibrillogenesis
XV	Multiplexins	Particularly in vascular, mesenchymal, neuronal, and epithelial basement membrane zones
XVI	FACIT	Cartilage, skin, intestine, heart, kidney, and arterial walls; structural integrity and in integrin-mediated signalling
XVII	Transmembrane	Adheres epithelial cells to the ECM
XVIII	Multiplexins	Basement membranes; Stability and signaling potential
XIX	FACIT	Various tissues; muscle development and differentiation
XX	FACIT	Corneal epithelium, tendon, cartilage, embryonic skin
XXI	FACIT	In collagen I abundant tissues
XXII	FACIT	Myotendinous junctions of the heart, skeletal muscle and cartilage; Force transmission during movement
XXIII	Transmembrane	Heart and retina; might be involved in integrin-mediated signaling
XXIV	Fibril-forming	Early stages of development, involved in collagen I synthesis
XXV	Transmembrane	Brain, spinal cord neurons and muscles; involved in myofiber development
XXVI	Beaded filament	Testes, ovaries and kidneys; might be involved in the reproductive system
XXVII	Fibril-forming	Involved in cartilage calcification, lung, ear and colon development
XXVIII	Beaded filament	Peripheral nerves
XXIX	Beaded filament	Skin, lung, colon, testis and small intestine; epidermal integrity

1.2 Collagen biosynthesis

Collagen turnover in muscles, tendons, and ligaments generally occurs at rates around 1-2% per day, depending on whether synthesis rates are measured in a postabsorptive state or after exercise (15, 16), and usually decreases significantly in elderly, as illustrated in 80+ year old people exhibiting a 68% reduction of procollagen compared to young adults (17). Notably, the fractional synthesis rates of collagen in tendons and muscles exhibit a pattern similar to the synthesis rates of myofibrillar and sarcoplasmic proteins up to 72 hours following a 1-hour exercise session, suggesting a coordinated adaptation of the musculotendinous system (18). To induce mRNA transcription of target collagen genes, mechanical loading is required which is detected by integrin transmembrane receptors. The interaction between collagens and cells is mediated by cell surface receptors such as integrins. These receptors monitor the integrity of the collagenous extracellular matrix by initiating processes of matrix degradation and renewal (19). Growth factors such as insulin growth factor-1 (IGF-1) and transforming growth factor-1 (TGF-1) are also released and bind to their corresponding receptors. Both processes result in intracellular signal transduction, allowing access to the nuclei (20). The mRNA transcribed from each gene undergoes processing and subsequently binds to the rough endoplasmic reticulum, the site of actual protein synthesis. Collagen is initially synthesized as its precursor, procollagen, which is linked via hydrogen bonds (21) and includes non-helical amino and carboxypropeptide extensions. As displayed in **Figure 1**, for collagen type I biosynthesis two α1 and one α2 chains are necessary. The aforementioned extensions increase the molecule's solubility, facilitating its movement within the cell for further modifications. The removal of the C and N propeptides by proteolysis (= procollagen peptidase; leading to “tropocollagen”) produces triple helical collagen molecules with short telopeptides at each end, which can assemble into highly ordered, string-like aggregates known as fibrils after vesicular transportation out of the fibroblast (22, 20). These cleaved N and C propeptides (for collagen type I) are accurately termed procollagen type I N-terminal propeptide (PINP) and procollagen type I carboxyterminal propeptide (PICP), respectively, and depict common collagen synthesis markers. In contrast, the carboxyterminal telopeptide region of type I collagen (ICTP) involved in cross-linking of collagen fibrils and interaction with other matrix components and found at the ends of the mature collagen molecule as described above is used as a marker of collagen I degradation (13, 23). During posttranslational processing, procollagen is translocated into the lumen of the endoplasmic reticulum, where it undergoes folding and further modifications (24). Posttranslational modifications include the hydroxylation of proline and lysine residues catalyzed by prolyl and lysyl hydroxylases, both demanding vitamin C and iron ions as cofactors (22).

The resultant cross-linked mature collagen fibers are eventually integrated in the extracellular matrix providing tissue stability.

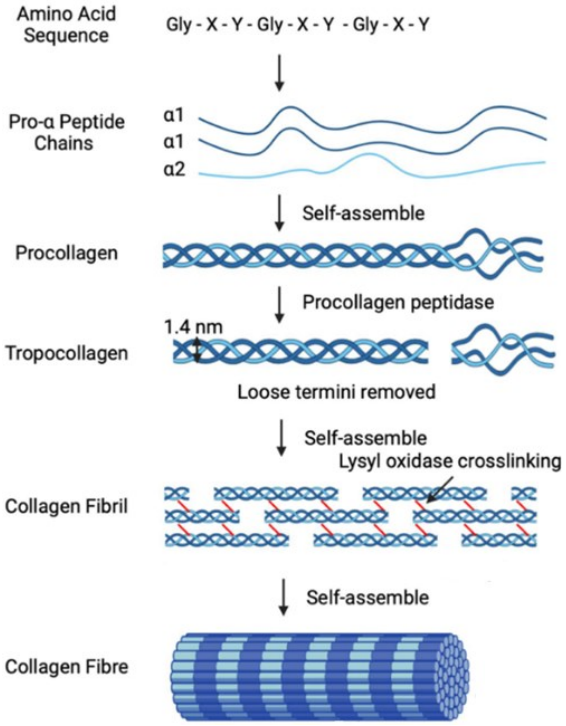


Figure 1: Collagen type I synthesis (modified from (21))

1.3 Collagen peptide manufacturing

Collagen peptides have gained remarkable attention in the past years, especially as a potential nutraceutical enhancing skin properties (25) and as a dietary supplement to support tissues required for proper movement execution (e.g. joints, ligaments). Owing to their broad and significant applications in the medical and food industries, several sources for collagen extraction have been established yet. Animal-derived collagen sources commonly exhibit porcine, bovine, chicken and marine, of which collagen type I, II and III are usually extracted (26, 27). Collagen peptides can be obtained by rather basic methods such as enzymatic and/or chemical hydrolysis as well as by means of ultrasound usage (28). Thermal treatments with temperatures over 40°C lead to collagen in their coiled triple helix form which is followed by hydrolysis (29). For chemical hydrolysis, organic acids such as lactic, citric and acetic acid of which the latter appears to be commonly used due to high yield and efficacy are applied. According to a comprehensive review, a majority of researchers stick to a 4°C incubation temperature along with a 0.5M concentration of mostly acetic or citric acid. Alkaline treatments with sodium or calcium hydroxide have also been implemented to remove redundant non-collagenous molecules (such as fatty acids) and diminish the activity of endogenous proteases (26). Proteolytic enzymes (“proteases”) facilitate proteolysis by breaking down triple-helical peptides into smaller peptide chains. A range of proteolytic enzymes, including pepsin, alcalase, trypsin, papain, chymotrypsin and pancreatin are known

to perform these catalytic reactions and choosing one specific enzyme depends on its ability to target certain peptide residues in the substrate (26). Both chemical and enzymatic extraction in combination have also been applied to obtain isolated collagen, thereby enhancing total yield while influencing the structure and properties of the product (30). The third commonly used extraction method depicts ultrasound, which induces mechanical, chemical and thermal alterations in the substrate through the creation and rapid collapse of cavitation bubbles resulting in shear forces that break the substrate's hydrogen bonding (26). A combination of different hydrolysis methods is often applied to enhance total yield. Accordingly, in a study comparing acid treatment only with combined acid and ultrasound treatment, collagen extraction yield improved with ultrasonic treatment, with the extraction rate rising significantly at higher ultrasonic amplitudes (31). By means of the aforementioned processes to denaturize and extract native collagen, hydrolyzed collagen is produced (**Figure 2**) which consists of small, low molecular-weight peptides even reaching weights less than 1 kDa depending on source and enzymes used for preparation (29, 32). As collagen and gelatin is often mistakenly used interchangeably, gelatin can be characterized by a partly hydrolysis of collagen fibers without dissolving in brine or water (33, 34) and is well known for its gelation property. Owing to environmental and economic reasons, there has been a recent shift toward plant-based collagen products. This is achieved by recombinant expression systems that have emerged as a promising alternative for producing exogenous human collagen, offering uniform collagen fibrils (35). Plant-based systems, particularly in tobacco, have been utilized to express recombinant human type I collagen (rhCOL1), reporting procollagen yields of up to 1 g/ kg of dry leaf weight (36). The produced collagen exhibits similar characteristic human native collagen by maintaining fibril-forming ability, heat stability (<40°C), protease resistance and supporting cell adhesion and expansion, making it suitable for tissue repair. Thus, plant-derived rhCOL1 seems to offer a viable biomaterial for future biomedical applications (35).

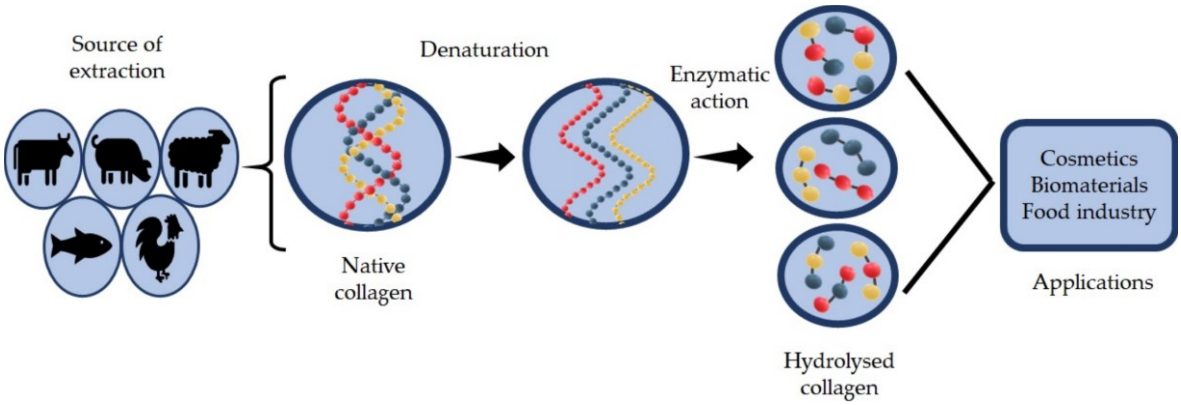


Figure 2: Overview of processing native collagen to small-molecular collagen peptides demonstrating diverse fields of applications (29).

1.4 Collagen peptide absorption and uptake kinetics

Absorption through the luminal brush boarder and subsequent entering of blood circulation mainly depends on the size/molecular weight of ingested collagen “types”. Gelatin as a partially hydrolyzed native collagen has been reported to overcome intestinal barrier by ~10%, but when adding pepsin-inactivating reagents, up to 50% of gelatin peptides could be absorbed properly (37). In order to unfold certain effects of bioactive peptides, a high amount of corresponding intact di-, tri- or oligopeptides need to pass enterocytes, which may be achieved by the following mechanisms (38): paracellular transport by enhanced tight junctions permeability, passive diffusion, endocytosis and carrier-mediated transport. The latter one uses H^+ as a carrier to transport di- and tripeptides through the intestinal endothelium, also known as peptide transporter 1 (PEPT1). As the first three mentioned absorption mechanisms have not yet been disclosed to be able to transport macromolecules in vivo, PEPT1 seems to play a pivotal role in intestinal peptide transportation. It also appears that peptides consisting of two to three amino acids can be transported, while the majority of larger peptides (tetra-, pentapeptides, etc.) are not, although a few have been absorbed in animal models (38). Peptide absorption via PEPT1 is influenced by several following factors (39). Competitive inhibition could alter the absorption of specific peptides in the presence of other co-existing compounds, as is the case outside of single peptide administration experiments. Their affinity to PEPT1 would then determine their extent of transportation. Total absorption may also be governed by transcriptional regulation of PEPT1. Upregulation of PEPT1 mRNA seems to be provoked by increased amino acid and protein intake and might also be downregulated by theaflavins (dimeric catechins found in black tea) (40). The maintenance of H^+ also positively affects intestinal peptide absorption and is improved by fatty acids and flavonoids by activating Na^+/H^+ exchanger, enabling sufficient proton flux. In the further course, ingested peptides face challenges to withstand cytosolic peptidases and hepatic first pass to finally reach peripheral blood circulation and deposit in target tissues. An in vitro study using gelatin extracted from sturgeon swim bladder demonstrated that 27% of the applied peptides (~0.4 kDa) were found following absorption in vitro (41). Similar results have been reported in an in-vitro study using a novel transwell co-culture of human intestinal epithelium cell line-6 (HIEC-6) and hepatic (HepG2) cells to more accurately simulate human in-vivo conditions. Herein, two different collagen hydrolysates showed corresponding peptide bioavailability of ~12-32% depending on the specific di- and tripeptides measured (42).

According to a recent review dealing with skin anti-aging effects, there are currently 25 specific collagen peptides that have been found in peripheral blood so far (43). Hydroxyproline (Hyp) appears to be the most abundant amino acid integrated in collagen-derived

peptides found in human blood, followed by the dipeptide Prolylhydroxyproline (Pro-Hyp). Pro-Hyp has also been reported to act as a growth-initiating factor for specific (p75NTR – positive) fibroblasts, which engage in wound healing (44). As the neurotrophin receptor p75NTR is also expressed in Pax7+ muscle satellite cells, human primary myoblasts and myotubes (45), a potential involvement of Pro-Hyp in muscle repair and myofibrillar recovery could be plausible.

Following the ingestion of fish-scale gelatin hydrolysate concentrate (0.385 g/ kg body weight) in water, eight other peptides (Ala-Hyp-Gly, Ser-Hyp-Gly, Ala-Hyp, Phe-Hyp, Leu-Hyp, Ile-Hyp, Gly-Pro-Hyp and Pro-Hyp-Gly) were quantified peaking within one to two hours in blood of healthy males (46). In another human trial, further collagen peptides (Pro-Hyp, Hyp-Gly, Pro-Ala, Pro-Ser, Gly-Pro, Gly-Pro-Pro, Gly-Pro-Ser, Gly-Pro-Lys and Gly-Pro-Gln) were identified in plasma with Ala-Hyp and Pro-Hyp being the most prevalent peptides within five hours (calculated by the area under the curve (AUC)) (47). Numerous other investigations reported characteristic uptake patterns and kinetics of ingested collagen peptides. A bolus of gelatin hydrolysate type I (9.4 g) from porcine skin led to maximum concentrations (Cmax) of free Hyp and peptides including Hyp in human blood sera after 30-60 min following a 12-hour fasting period, which was also demonstrated in another human trial administering 8 g of collagen hydrolysate (48). In the second experiment, 23 g/ 60 kg bodyweight of chicken feet hydrolysate (type I) and 11g/ 60 kg bodyweight of chicken cartilage hydrolysate (type II) elicited Cmax after 60-120 min for both products. In both experiments, the ratio of free Hyp to Hyp-containing peptides in human blood stayed 3:1 (49). 20g of powdered collagen, hydrolyzed collagen peptide powder and liquid collagen from different manufacturers resulted in similar Cmax of essential amino acids, even compared to bone broth and hydrolyzed casein (but not when AUC was considered), peaking 30-50 min following administration. Proline and glycine Cmax also exhibited similar levels within the collagen products with similar peak uptake patterns ranging from 40-70 min, but leucine Cmax was significantly lower than that of dairy protein supplements (all peaking within 30-50 min) (50). Moreover, the ingestion of 80 mg/ kg bodyweight collagen tripeptides yielded significantly higher plasma levels of Gly-Pro-Hyp, Gly-Pro, Pro-Hyp and Hyp-Gly within four hours than collagen samples containing 51% or 2% tripeptides. Interestingly, Gly-Pro Cmax was reached after four hours displaying a stark contrast to the absorption of other peptides, which might be caused by degradation of Gly-Pro-X peptides or difficulties in intestinal transportation (51, 52). Instead and due to the relatively high Cmax, Pro-Hyp appears to be a rapidly produced metabolite of Gly-Pro-Hyp. According to the additionally performed measurement of urinary excretion, significant amounts of Gly-Pro-Hyp may remain in intact form following collagen tripeptide ingestion, highlighting its stability (51). In a rat study, the

peroral and intraperitoneal administration of a collagen tripeptide preparation (~ 90% accounted to tripeptides) extracted from porcine skin also led to highest four-hour AUCs of Gly-Pro-Hyp among all measured tripeptides, followed by substantially lower, rather negligible, levels of Gly-Pro-Ala and Gly-Ala-Hyp. Strikingly, dipeptide levels of Pro-Hyp even excelled the ones of Gly-Pro-Hyp (also peaking within 30 min as Gly-Pro-Hyp did), followed by 10-100 times lower AUCs of Gly-Pro and Ala-Hyp, respectively, once more indicating the rapid degradation of Gly-Pro-Hyp to predominantly Pro-Hyp. X-ray images further revealed that labeled Gly-[^{3}H]Pro-Hyp majorly accumulated in the periosteum, bones and skin within 24 hours, emphasizing the ability of collagen tripeptides to be incorporated into connective tissues (53). Beyond that, even some oligopeptides might be absorbed (perhaps by passive intestinal diffusion) and resist to serum peptidases to a certain extent, at least in rats (54). Although evidence is still lacking, these oligopeptides could potentially be further degraded into tri- and dipeptides, thereby prolonging the period of elevated concentrations and improving the sustained supply of specific bioactive peptides.

Preliminary evidence with respect to the type of hydrolysis of collagen points to superiority of enzymatic compared to non-enzymatic hydrolyzed collagen to enhance postprandial absorption. 35 g of both types showed a significantly higher AUC human plasma concentration of glycine, proline, hydroxyproline and Gly-Pro-Hyp in favor of enzymatically hydrolyzed collagen (55). A relatively long-term administration regimen of four weeks was carried out in women around 20 and 60 years old, with a daily intake of 4.5 g of collagen hydrolysate derived from porcine skin. Ahead of each daily ingestion, free hydroxyproline levels were significantly higher than those before the trial. This suggests that the elevated Hyp-containing peptides returned to baseline levels within 24 hours, while free Hyp levels might have remained elevated beyond 24 hours due to degradation of Hyp-peptides. Furthermore, free Hyp and Hyp-containing peptides AUC did not differ between older and younger participants (56).

Collagen peptide uptake also exhibits a dose-dependent pattern. It has been reported that 25 g/ 65 kg/ body weight collagen hydrolysate elicited significantly higher free Hyp and Hyp-peptide concentrations in human plasma after one to two hours than doses of 2 g and 10 g / 65 kg/ body weight. The AUC of free Hyp was significantly higher at 25 g than 2 g and 10 g (4.3 and 1.4 times, respectively) whereas the AUC of Hyp-peptides did not differ significantly between 25 g and 10 g (57). In an acute exercise intervention trial, blood from resistance trained men showed significantly more elevated glycine, proline and hydroxyproline levels over a seven hour-period following the ingestion of 30 g hydrolyzed collagen than 15 g and 0 g combined with 40 repetitions of barbell squats. Similar results were observed for the collagen synthesis marker PINP (as the concentration AUC) (58), suggesting enhanced uptakes in a dose-response manner.

The absorption and in-vivo detection of collagen peptides are also highly dependent on the molecular weight (distribution). In a study administering three gelatin products, the low-molecular weight gelatin hydrolysate (LMWGH) (<3 kDa) included significantly higher free Hyp levels and total amino acids compared to gelatin (>100 kDa) and gelatin hydrolysate (>3 kDa). The four hour-AUC of free Hyp and Pro-Hyp, but not Hyp-Gly was significantly more elevated than in both other products (59). In a rat experiment, high-molecular weight collagen peptide (HCP) (~2 kDa) from fish scales provoked significantly lower plasma concentrations of Gly-Pro-Hyp and Pro-Hyp than a collagen tripeptide preparation (CTPH) (~0.5 kDa) and its derived lower-weighted preparation (CTPL) (~0.25 kDa). The Gly-Pro-Hyp six hour-AUC values in the CTPH and CTPL groups were 2.6 and 12 times higher than in the HCP group, respectively. The AUC values of Pro-Hyp were also 1.5 and 3.4 times higher, respectively. Gly-Pro-Hyp and Pro-Hyp as well demonstrated strong resistance to chemical and enzymatic degradation in gastric juice, gastrointestinal enzymes, and plasma (60). Furthermore, in-vitro results showed a significantly higher proportion of small peptide oligomers below 0.5 kDa in a X-Hyp and X-Hyp-Gly enriched collagen peptide (CP) product (<2 kDa) compared to a normal collagen hydrolysate (~ 70% vs. 50%) following digestion. The CP product also demonstrated greater peptide uptake (27%) than the hydrolysate (21%). These findings underscore the potential use of Hyp-containing, low molecular weight collagen peptides in (food) supplements to provide high yields of specific collagen peptides in corresponding tissues after ingestion (61).

The source of collagen-derived peptides may also play a substantial role in effective absorption. This is underlined by a crossover investigation in healthy males ingesting gelatin hydrolysate from either fish scale, fish skin or porcine skin, all of which had a molecular weight of ~5 kDa. The results of the 24 hour concentration AUCs suggested the fish scale preparation to be superior in enhancing Hyp-peptide levels whereas the fish skin hydrolysate elicited significantly lower levels of free Hyp than fish scale and porcine skin products (62). This might be somewhat surprising since collagen from fish skin usually comprises less proline and hydroxyproline compared to mammalian sources (63). Hence, future trials are encouraged to investigate source depending uptakes of specific bioactive collagen peptides. Although absorption kinetics were not considered, a study utilizing bioinformatics tools to classify collagen from various animal sources based on their physicochemical properties and other characteristics identified potential bioactive peptides. The analysis suggested that collagen from bovine and porcine sources is similar and can be used interchangeably, while trout and salmon collagen also share similarities. Chicken collagen, although closer to bovine and porcine, was the most distinct among the species studied (64). Based on these

findings, it appears that certain differences in collagen peptide bioactivity exist among terrestrial species, suggesting the need for further investigations to better understand species-specific peptide functions.

1.5 Exercise induced collagen synthesis

While the previous chapter thoroughly explained how ingested collagen preparations and their degraded peptides enter the bloodstream in fasted and resting individuals, the next section will focus on the collagen synthesis responses triggered by exercise. Several studies have examined the impact of specific training modes on protein synthesis in muscle and tendon connective tissue, with the latter acting as a force transmitter between the myotendinous junctions and the bones. In an animal experiment, rats were subjected to either concentric, eccentric, or isometric training for four days. Results showed significantly increased gene expression of collagen type I, III, transforming growth factor β-1 (TGFβ-1, a mediator of mechanically induced collagen synthesis), and lysyl oxidase, a copper-dependent enzyme that catalyzes the cross-linking within and between collagen (chains), in the Achilles tendon and gastrocnemius muscle. Notably, mRNA expression of tissue inhibitors of metalloproteinases 1 & 2 (TIMP, inhibit matrix-degrading enzymes), Col I, and TGFβ-1 were significantly higher in the eccentric training group than in the concentric training group, with muscle tissue appearing more sensitive to the type of contraction than tendinous tissue (65). Another rat study reported an increased expression of mechanically sensitive mechano-growth factor (MGF/ IGF-1Ec) and insulin like growth factor-1 isoforms IGF-1Ea following four days of exercise. Both markers are splice variants of the IGF gene (66). IGF-1 is recognized as a potent growth factor stimulating collagen synthesis (67) and may thus be considered an indirect marker. In the aforementioned study, both markers (isoforms of IGF-1) did not show significant differences between concentric, eccentric, and isometric training in tendons. However, in muscle tissue, eccentric training elicited the greatest changes (68). Similarly, a human one-time training intervention found that elevated collagen synthesis was independent of whether the exercise involved unilateral lengthening or shortening contractions within 8.5 hours. However, myofibrillar protein synthesis was stimulated to a greater extent by eccentric movements. (15). A longer-term study involved both young and older males (approximately 24-69 years old) who exercised three times a week for eight weeks, using either concentric or eccentric movement-based training. Patella tendon biopsies revealed significantly increased fractional synthetic rates of protein synthesis in both concentric and eccentric groups after four weeks, but no differences existed after eight weeks compared to the control group. The proximal Patella tendon cross-sectional area significantly increased after eight weeks in both groups (only in younger subjects). Intri-

guingly, gene expression of COL1A1, COL6A3, COL12A1 (collagen genes for their respective alpha chains), TIMP1 and TGFβ-1 were significantly elevated after four weeks in the eccentric group compared to the concentric one, at least in young males (69), indicating that lengthening contractions stimulate growth factors, collagen degradation inhibitors and specific stabilizing collagen proteins effectively. Eccentric unaccustomed knee extension bouts alone have further shown to stimulate endomysial collagen type IV 22 days post-exercise in human vastus lateralis together with reciprocal increases in serum TIMP and matrix metalloproteinase (MMP, matrix-degrading enzymes) (70). These findings suggest prolonged ECM remodeling mainly provoked by eccentric contractions.

Irrespective of contraction type, a human trial involving young men performing one-hour kicking exercise bouts demonstrated similar patterns of tendon collagen, muscle collagen, myofibrillar protein and sarcoplasmic protein fractional synthetic rates within 72 hours following exercise (18). Collagen synthesis in human tendon and muscle tissue reaches peak rates at 24 hours and remains significantly elevated at 48 hours, suggesting a coordinated adaptation with myofibrillar protein synthesis. However, muscle protein synthesis rates were approximately twice as high as those for collagen, indicating comparatively slower but steady growth and development of collagen-containing tissues.

1.5.1 Sex-specific differences in collagen synthesis following exercise

Hormonal differences between males and females have been reported to possibly influence collagen synthesis. Estrogen, a steroid produced in ovaries, testes, and adrenal cortex, typically refers to 17β-estradiol and is usually found abundantly in women. By binding to its receptors, including estrogen receptor α (ERα), ERβ (both intracellularly located), and G-protein-coupled estrogen receptor 1 (GPER1), which are the most prominent and have the highest affinity, estrogen exerts several physiological functions related to health and disease (71). ERs convey estrogen signaling and regulate the transcription of genes involved in growth, proliferation, differentiation, and various other cellular processes (72). ERs have been found in human skeletal myofibres & endothelial cells (73), ligaments (74) (also via mRNA expression (75)) and indirectly detected via gene expression in fibroblasts of tendons (76). In a cross-sectional investigation, females habituated to distance running (>40 km weekly) failed to demonstrate advanced Achilles and Patella tendon hypertrophy compared to age-matched non-active peers. When weight-normalized cross-sectional area (CSA) was compared, male runners showed significantly more augmented CSA than female runners, which might be influenced by the hormonal status according to the authors (77). Another trial reported healthy women's resting and post-exercise Patella tendon fractional synthetic rate to be lower than those of age-matched healthy males and that the phase of menstrual cycle did not influence collagen synthesis (78). Moreover, myofibrillar and intramuscular

collagen synthesis (measured by means of vastus lateralis biopsies) may as well not be controlled by specific menstrual cycle phases in both resting and exercised conditions (79). Another study solely including females either receiving oral contraceptives (estradiol and progestogen abundant) or being in the follicular phase (typically presenting low levels of both hormones) without supplementation were tested whether estradiol concentrations contribute to acute musculotendinous adaptations. Baseline and 24 hours following exercise (one hour of repeated unilateral leg kicks) values of Patella tendon collagen synthesis were higher in the follicular group than in the contraceptive one. The follicular group also displayed a significant rise in muscle collagen synthesis whereas the oral contraceptive supplementing subjects did not. In addition, serum, tendon & muscle dialysate IGF-1 concentrations were significantly elevated whereas IGF-1 binding proteins (IGF-1BP 1, 3, 4) were significantly more abundant in the contraceptive group, depicting lower growth potential due to scarce IGF-1 bioavailability (80). In contrast, postmenopausal women receiving long-term estradiol replacement therapy (ERT) showed higher resting and post-exercise Patella tendon synthesis rates compared to aged-matched non-users. However, tendon force and Young's modulus (a measure of deformation resistance) were significantly lower in the ERT group. Overall, this suggests insufficient cross-linking between collagen molecules resulting in diminished tendon mechanical and material properties in an estradiol rich milieu (81). Decreased enzymatic cross-linking of collagen has already been shown in an engineered ligament model treated with estrogen by reduced lysyl oxidase activity of 77% (48 hours after estradiol administration) (82). It was also reported in 113 international soccer players that tendon injuries occurred nearly twice as much in the late follicular phase than in the early follicular or luteal phase (83). Moreover, knee laxity and anterior cruciate ligament rupture are linked to estrogen levels (84), illustrating a sex-specific overall higher exposition to sports-related injuries in collagen abundant tissues (e.g. tendons, sinews, myotendinous junctions).

Lastly, findings from a recent case report will be presented, as it is the only study to date investigating the combination of exercise and collagen ingestion, along with estrogen concentrations, on collagen synthesis. In this double-blind, randomized crossover single-case study, serum of a female CrossFit athlete during late follicular phase (highest estrogen levels) disclosed 24% and 40% reduced PINP (collagen synthesis marker) levels compared to the onset of menstrual cycle (usually low estrogen levels) ingesting 0 g and 30 g of hydrolyzed collagen (HC) following bouts of back squats. HC enhanced PINP concentrations during both menstrual phases, with nearly double the increase in the low estrogen condition, indicating estrogen/ menstrual cycle-dependent collagen synthesis deficiencies (85).

1.6 Combined exercise and collagen peptide supplementation provoked effects on collagen synthesis

It is nowadays well established that combining nutritional intake with exercise, in particular with resistance training (RT), exerts synergistic effects on muscle protein synthesis. While an amino acid-rich, nutritional supplementation alone usually evokes peak synthesis responses within one to two hours, adding acute bouts of resistance exercises may prolong the "muscle-full" effect even beyond 24 hours (86). Hereafter, findings from human trials investigating the synergistic effects of exercise plus protein/ collagen peptide supplementation on collagen synthesis will be presented.

Recreational young male and female athletes participating in a double-blind randomized controlled trial either ingested a single dose of 30 g of whey, collagen protein or a metabolically inert placebo following six sets of barbell squats. Biopsies from the vastus lateralis revealed no significant differences in myofibrillar and muscle connective protein fractional synthetic rates in the first five hours after exercise in the collagen group compared to the others. The authors concluded that muscular collagen synthesis might be substantially higher in tissues with high collagen content, such as tendons, since proline and glycine requirements would also be increased (87). Muscle collagen protein synthesis in older women (~69 years old) did not significantly differ at rest and following RT when administering 30 g of whey or collagen protein whereas myofibrillar protein synthesis was significantly more pronounced with whey in a rested and exercised state (88). Another recently conducted trial including older men supplementing either a mix of 20 g HC plus milk protein or 20 g of a non-blended milk protein demonstrated similar increases in vastus lateralis myofibrillar protein synthesis four hours after ingestion and combined ingestion with RT between groups (89). These results indicate that partial replacement of milk protein with collagen protein might also be an adequate stimulus for providing sufficient muscular collagen synthesis, at least in older subjects.

Only two studies exist at the moment investigating dose-response effects of collagen supplementation in combination with plyometric or RT on collagen synthesis. The first one used an in-vitro approach by treating engineered ligaments (cultured with anterior cruciate ligament fibroblasts) with human blood, which had been collected before and after rope-skipping bouts with previously taken 5 g or 15 g of vitamin C enriched gelatin. Collagen content in the ligament graft significantly elevated in the 15 g group whereas collagen concentration significantly increased in the 5 g and 15 g group. PINP levels significantly increased only after 15 g gelatin (90). In the other double-blind, randomized controlled cross-over trial, resistance-trained men were administered 0, 15 or 30 g of HC mixed with vitamin C and performed bouts of back squats to induce collagen synthesis. The 30 g group was the only to induce significant increases in PINP levels (AUC) within six hours after the RT

session. This result might have been induced by higher concentrations of glycine, proline and hydroxyproline in the 30 g group compared to the 15 g (58).

1.6.1 Relevance of adding vitamin C

As mentioned in chapter 1.2, both prolyl and lysyl hydroxylases demand vitamin C (ascorbic acid) as a cofactor to produce functional collagen molecules. Both enzymes post-translationally modify proline and lysine residues intracellularly (91). Therefore, vitamin C is deliberately added to collagen supplementation in some studies (92, 93) to enhance collagen synthesis. A systematic review on the efficacy of added vitamin C provides evidence from preclinical trials that ascorbic acid may propel bone healing after fractures and augment collagen type I synthesis. Nevertheless, the available data are limited and do not provide a clear understanding of musculoskeletal injuries, as preclinical findings have not been consistently replicated in clinical trials (94). Vitamin C intake and blood status reported in European studies refer to low deficiency rates with the majority of the population reaching dietary guidelines (95). Based on these findings, adding ascorbic acid to possibly maximize effects of collagen supplementation currently lacks evidence and yet no single study has examined whether collagen supplementation plus vitamin C is superior to collagen supplementation alone in a double-blind manner. Thus, future investigations are encouraged to implement both such groups and directly measure collagen synthesis in exercised tissues like tendons and muscles. This should be done by means of tissue biopsies rather than collecting PINP via blood samples, as the latter is also influenced by systemic collagen synthesis and hence does not obtain local effects (96). A reference method for PINP, which has also been used extensively as a bone formation marker, to standardize/ harmonize available assays is needed as well (97). Furthermore, liver and kidney function (98) as well as nutritional status, macro- and micronutrient intake in close proximity to measurement might also influence this marker (99).

1.7 Collagen peptide supplementation induced molecular responses

Only a few studies have been conducted to investigate the molecular mechanisms triggered by collagen peptide administration (with respect to musculotendinous tissues), going beyond just phenotypic adaptations. As collagen protein is typically low in branched chain amino acids, particularly in leucine, a potent driver of muscle protein synthesis (100), it seems somewhat surprising that hydroxyprolyl-glycine (Hyp-Gly) has already shown to trigger myotube hypertrophy. This collagen derived dipeptide promoted differentiation of myoblasts through the phosphoinositide 3-kinase/ protein kinase B/ mammalian target of rapamycin (PI3K/Akt/mTOR) pathway and increased myotube size in murine C2C12 my-

oblasts (101). Another in-vitro experiment using mouse tendon cells demonstrated prolylhydroxyproline (Pro-Hyp) to promote tendon cell differentiation, maturation and proliferation with significantly enhanced phosphorylation of extracellular signal–regulated kinase (ERK; its cascade is crucial in regulating cell cycle progression), and stimulated extracellular matrix production and collagen type I organization. The dipeptide also increased cell motility and migration, along with extended lamellipodial protrusions and more active β1-integrin-comprising focal contacts (102). Following 21 days of daily administration, Pro-Hyp also influenced the TGFβ-1/ Smad (mothers against decapentaplegic homolog 3 or SMAD family member 3) signaling pathway, which is known for regulation of tissue remodeling and tissue homeostasis (103, 104). In more detail, phosphorylated Smad3 (a protein substantial for gene transcription after receiving TGFβ-1 signals from cell's surfaces) positive cells increased significantly around granulation and injured muscle tissue in mice. Additionally, muscle regeneration in granulation tissue was linked to less fibrosis in the Pro-Hyp group (105). Similar findings were observed in a mouse study applying dexamethasone to induce muscle atrophy. Smad2, also a target of TGFβ-receptors but inversely regulating TGF-β autoinduction compared to Smad3 (at least in dendritic cells (106)), was found to be significantly suppressed in its phosphorylated (activated) form while TGF-β gene expression was unaffected in muscle tissue after four weeks of collagen peptide administration. Furthermore, mRNA expression of the muscle growth controlling and limiting myostatin was significantly inhibited whereas Akt/ mTOR signaling pathway was significantly more phosphorylated and thus activated, indicative of muscle preservation (107).

So far, two trials have been conducted in human subjects each investigating acute or chronic molecular responses to exercise combined with collagen peptide supplementation. The short-term trial referred to significantly upregulated PI3K/ Akt and mitogen-activated protein kinase (MAPK) (and insignificantly elevated mTOR) anabolic pathways in recreationally active men following intense leg extensions and acute 15 g collagen peptide intake. These effects were evident at the four-hour measurement, but not at the one-hour or 24-hour time points, suggesting a rather small time window of collagen administration-related skeletal muscle anabolic signal transduction (108). The long-term (12-week) RT experiment including physically active males daily ingesting 15 g of collagen peptides investigated effects on the human skeletal muscle proteome (derived from vastus lateralis biopsy). In total, 221 and 44 proteins were upregulated in the collagen and placebo group, respectively. Collagen peptide intake significantly enhanced signal transduction of MAPKs and Akt indicating muscle hypertrophy effects and metabolic health. Heat-shock transcription factor (HSF1) activation and dependent transactivation was also highly augmented (fold enrichment of 40 and 32, respectively) (109). HSF1 has been proposed to play a pivotal role in the recovery process of injured skeletal mice muscles via a higher population of Pax7+

satellite cells and decreased pro-inflammatory cytokines (110). Additionally, fibronectin showed a ~2.2 higher fold change in the collagen group disclosing an involvement in cellular repair since fibronectin has been reported to trigger satellite cell expansion in recovering muscle tissue (111).

1.8 Intentions and hypotheses of the dissertational project

Several short- and long-term human trials involving collagen peptide supplementation combined with physical exercise have been conducted so far, mainly focusing on possible adaptations with respect to body composition, muscle and tendon apparatus, aerobic and anaerobic endurance performance, strength and regeneration. Since recovery-associated experiments in the related field did not involve long-term supplementation with accompanying exercise studies, the goal of this doctoral research project was to implement such a design and determine whether prolonged regimens could elicit similar or greater regenerative capacity. A brief overview of the results of the aforementioned studies will be provided before presenting the hypothesis.

The first trial to be conducted with an appropriate design was a pilot study, including recreationally active males and females supplementing 3 g of hydrolyzed chicken sternal cartilage extract (“BioCell Collagen”) for six weeks. Due to low sample size (n = 8), statistical significance was not calculated, but descriptive statistics hint to beneficial effects of the supplement, conveyed by decreased surges of muscle damage markers creatine kinase (CK), lactate dehydrogenase (LDH) and inflammatory marker C-reactive protein (CRP), discomfort and movement pain scores and blunted decreases in bench-press performance (112). Another trial supplementing 10 g collagen peptides daily for four weeks without accompanied exercise intervention in healthy male adults led to significantly reduced immediate muscle soreness and enhanced 48 hour post exercise muscle strength (113). In a one-week trial implementing a similar cohort without exercise intervention, 20 g of collagen peptides mixed with 80 mg vitamin C caused countermovement jump height to be significantly higher 48 hour post exercise compared to the placebo group, while soreness, pain, muscle damage and pro-inflammatory markers, PINP and immunological cell types remained quite similar between groups (114). At last, RT-experienced males consuming 15 g for 12 days and exercising at least three times a week showed an enhanced countermovement jump height after 24 hours but failed to demonstrate changes in soreness, synthesis/ degradation blood markers and maximal strength (115).

The four aforementioned studies had in common that a muscle-damaging unaccustomed exercise, usually so-called drop jumps, preceded the following 24-hour wise indirect measurements of versatile recovery-related markers. Moreover, muscle-damaging bouts were performed only once, rather than twice, which would have accounted for additional

time effects in comparing pre- and post-supplementation results. Another limitation appears to be the lack of prolonged (resistance) exercise alongside collagen peptide administration as the synergistic effect of exercise and protein is well known to promote beneficial musculotendinous adaptations, which typically require around three months to occur (116–118). Based on these shortcomings, a twelve-week concurrent training (combining both endurance and resistance training elements) accompanied with 15 g of collagen peptide intake in low to moderately active males has been conducted as part one (paper 1 & 2) of the research project. Of particular interest were isokinetic dynamometer and force plate derived data (maximal voluntary contractions, rate of force development and countermovement jump height), body composition data (fat- and fat-free mass) as well as blood derived markers (CK, LDH, myoglobin (MYO) and CRP). The corresponding research design and results are illustrated in detail in section 2, paper 1 (119) and 2 (120). As shorter-lasting trials have already reported effects in these regards, *it was assumed that prolonged collagen peptide intake alongside regular exercise may enhance regenerative capacity evinced by favorable changes in such parameters.*

Part 2 of the doctoral project initially exhibited a long-term dose-response study in order to elucidate the optimal intake dose to evoke enhanced recovery. Unfortunately, economic issues prompted to “steer” in another direction, which depicted carrying out a systematic review with meta-analysis about possible prolonged collagen peptide supplementation-related adaptability of body composition, the musculotendinous apparatus, maximal strength and recovery. Details of this comprehensive analysis are displayed in section 2, paper 3 (121). A brief overview of the rationale and the relevant literature is provided below, leading into the hypotheses.

Data of an unpublished meta-analysis (Bischof et al. 2024), which was part of a poster presentation in Freiburg, Germany, hint to collagen peptide-induced changes in body composition. Moderately trained young to untrained old participants (n = 273) performing 12 weeks of resistance training, three times a week, together with daily ingestion of 15 g collagen peptides were included (109, 122–125). The results revealed a statistically significant average reduction of fat mass by 0.9 kg and an increase in fat-free mass by 1.2 kg following the interventions. However, two studies implementing concurrent training for 12 weeks with the same amount of collagen intake have not found any significant changes with respect to fat-free mass (119, 126).

Tendon morphological and mechanical adaptations following long-term collagen peptide intake have already been investigated in a limited number of exercise trials. Equivocal findings have been reported: two studies observed significantly enhanced Patella and Achilles tendon cross-sectional area (CSA) (9, 10), Young’s modulus and stiffness (127), while another one, involving a 15-week resistance training regimen, detected no changes

at all compared to a placebo (128). A stiffer and larger (greater CSA) tendon may offer enhanced injury preventive functions as higher forces can be applied without tendon material-related damage. Therefore, the meta-analysis also examined the potential of collagen intake to adapting tendon properties, which may particularly of practical use for athletes exposed to high force-bearing demands on their tendons.

Gains in maximal strength and muscle size are usually indicative of improved metabolic health and a reduced risk of comorbidities (129). Whether the use of collagen peptides may also contribute to such favorable conditions, has been the subject of previous studies. Increases in muscle size were observed in some (9, 130), but not all long-term trials with an accompanying training intervention compared to an ingestion of placebo (125, 131). Maximal strength, which is frequently gauged as maximum voluntary contraction during knee extensions by a dynamometer, has also been detected to be increased (122, 123) or unchanged (93, 127) compared to a placebo group.

Lastly, muscular strength-specific recovery has been measured by previously mentioned studies (29, 113–115) (see second paragraph of section 1.8). Briefly, collagen intake evoked increases in maximal and reactive strength as well as decreases in muscle soreness. However, the causes of these changes remain largely unknown since the duration of the supplementation intervention was too short and/ or not accompanied by exercise. Paper 1 contributed our findings to the recovery-specific literature by addressing the aforementioned shortcomings. However, a systematic analysis of the effects of recovery-related studies in the context of collagen supplementation was still lacking.

Based on the above-mentioned findings, *it was supposed that long-term collagen peptide administration might statistically significantly enhance adaptations regarding body composition, muscle architecture and tendinous properties, maximal strength and regenerative capacity.*

2. Published articles

1. Bischof, K., Stafilidis, S., Bundschuh, L., Oesser, S., Baca, A., & König, D. (2023). Influence of specific collagen peptides and 12-week concurrent training on recovery-related biomechanical characteristics following exercise-induced muscle damage - A randomized controlled trial. *Frontiers in Nutrition*, *10*, [1266056]. https://doi.org/10.3389/fnut.2023.1266056
2. Bischof, K., Stafilidis, S., Bundschuh, L., Oesser, S., Baca, A., & König, D. (2024). Reduction in systemic muscle stress markers after exercise-induced muscle damage following concurrent training and supplementation with specific collagen peptides – a randomized controlled trial. *Frontiers in Nutrition*, *11*, [1384112]. https://doi.org/10.3389/fnut.2024.1384112
3. Bischof, K., Moitzi, A. M., Stafilidis, S., & König, D. (2024). Impact of Collagen Peptide Supplementation in Combination with Long-Term Physical Training on Strength, Musculotendinous Remodeling, Functional Recovery, and Body Composition in Healthy Adults: A Systematic Review with Meta-analysis. *Sports medicine (Auckland, N.Z.)*. https://doi.org/10.1007/s40279-024-02079-0

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Influence of specific collagen peptides and 12-week concurrent training on recovery-related biomechanical characteristics following exercise-induced muscle damage—A randomized controlled trial

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Introduction: It has been shown that short-term ingestion of collagen peptides improves markers related to muscular recovery following exercise-induced muscle damage. The objective of the present study was to investigate whether and to what extent a longer-term specific collagen peptide (SCP) supplementation combined with a training intervention influences recovery markers following eccentric exercise-induced muscle damage.

Methods: Fifty-five predominantly sedentary male participants were assigned to consume either 15 g SCP or placebo (PLA) and engage in a concurrent training (CT) intervention (30 min each of resistance and endurance training, 3x/week) for 12 weeks. Before (T1) and after the intervention (T2), eccentric muscle damage was induced by 150 drop jumps. Measurements of maximum voluntary contraction (MVC), rate of force development (RFD), peak RFD, countermovement jump height (CMJ), and muscle soreness (MS) were determined pre-exercise, immediately after exercise, and 24 and 48 h post-exercise. In addition, body composition, including fat mass (FM), fat-free mass (FFM), body cell mass (BCM) and extracellular mass (ECM) were determined at rest both before and after the 12-week intervention period.

Results: Three-way mixed ANOVA showed significant interaction effects in favor of the SCP group. MVC ($p = 0.02$, $\eta p^2 = 0.11$), RFD ($p < 0.01$, $\eta p^2 = 0.18$), peak RFD ($p < 0.01$, $\eta p^2 = 0.15$), and CMJ height ($p = 0.046$, $\eta p^2 = 0.06$) recovered significantly faster in the SCP group. No effects were found for muscle soreness ($p = 0.66$) and body composition (FM: $p = 0.41$, FFM: $p = 0.56$, BCM: $p = 0.79$, ECM: $p = 0.58$).

Conclusion: In summary, the results show that combining specific collagen peptide supplementation (SCP) and concurrent training (CT) over a 12-week

period significantly improved markers reflecting recovery, specifically in maximal, explosive, and reactive strength. It is hypothesized that prolonged intake of collagen peptides may support muscular adaptations by facilitating remodeling of the extracellular matrix. This, in turn, could enhance the generation of explosive force.

Clinical trial registration: ClinicalTrials.gov, identifier ID: NCT05220371.

KEYWORDS

specific collagen peptides, recovery, muscle damage, concurrent training, repeated bout effect

1. Introduction

As an integral component of connective tissue, collagen accounts for approximately 30% of the total protein mass in the human body (1). In contrast to the amino acid composition of myofibrillar protein, collagen peptides (CP) mainly consist of glycine, proline and hydroxyproline. Collagen is found in various tissues such as cartilage, tendons, ligaments and muscles. It not only provides stability and protection, contributing to the structural integrity of these tissues, but also plays a crucial role in enhancing force transmission. There is a constant process of collagen degradation and synthesis to perform these functions, with a turnover rate of about 0.5–2% per day. This process is influenced by incoming signals from the surrounding extracellular matrix (2, 3). In addition, muscles play a vital role in remodeling intramuscular connective tissue (IMCT), an essential component of the extracellular matrix (ECM). A remodeling process that involves binding skeletal muscle-specific α7β1-integrin to high-affinity ligands such as laminin, fibronectin, and collagen. This interaction triggers signal transduction pathways that subsequently activate the appropriate gene expression patterns, leading to the development of ECM plasticity (4). An optimal tailored and distinct IMCT in which collagen types I and III account for ∼75% (5) is essential for proper mechanotransduction and, thus, optimal movement performance.

Several studies have investigated the influence of specific exercise modalities on the protein synthesis of muscle and tendon connective tissue, the latter acting as a force transmitter between myotendinous junctions and bones. Muscles, tendons, and IMCT of the lower limbs were found to have higher gene expression of certain collagenous tissues and mediators (e.g., collagen type I, III, TGFβ-1, lysyl oxidase) after both resistance and endurance training in rodents and humans, suggesting a possible role in regulating muscle adaptation, repair, recovery and restructuring (2, 5–8). Muscle fiber hypertrophy is not always accompanied by equal turnover rates of ECM components, suggesting different growth potential (9). Recently, supplementation with CP was shown to stimulate collagen synthesis (10). In contrast, the intake of other proteins (e.g., essential amino acids, whey) did not significantly increase MCT synthesis rates, even when combined with exercise (11–14).

The ability to generate force decreases markedly after exercise-induced muscle damage, which persists for up to 7 days (15). In addition to physical treatments (10), nutritional supplementation with various protein sources has been linked to a small association between muscle function recovery, muscle soreness, and indirect markers of muscle damage (16). Recently, CP administration has resulted in significant improvements in handgrip strength (17) and isokinetic quadriceps strength (18), producing similar results to whey protein (19).

Both endurance and strength exercises can induce muscle damage, resulting in local cellular and systemic responses (20). Delayed onset muscle soreness (DOMS), a commonly used marker of muscle damage, usually increases from 8 h, peaks at 24–48 h after exercise (21) and is easily detected. Further research is needed to provide a clear picture of the potential benefits and efficacy concerning recovery from high-intensity exercise after CP supplementation. Four studies (including two pilot studies) have shown a non-significant flattening of the creatine kinase (CK) curve (22), a significant increase in countermovement jump height (CMJ) at 24 h (23) and 48 h (24) and MVC at 48 h (25) after a muscle-damaging exercise bout. Apart from the acute changes, a three-week power training intervention with a daily intake of 20 g CP showed an attenuated decline in RFD during maximal isometric contraction and an improved eccentric deceleration impulse during CMJ compared to the placebo group (26).

Based on the aforementioned findings, there is limited data on recovery from both long-term supplementation and training interventions. Therefore, the objective of the present study was to investigate whether daily CP supplementation is superior to placebo in promoting recovery after a 3-month training intervention. To this end, data from performance tests such as MVC, RFD, and CMJ and perceived muscle soreness will be collected that may provide insight into potential myofibrillar and IMCT adaptations that could indicate improved recovery.

2. Materials and methods

2.1. Experimental design and participants

The study was conducted as a single-center, double-blind, placebo-controlled, randomized, prospective trial at the University of Vienna, Austria. Before recruitment, the study was approved by the Ethics Committee of the University of Vienna (reference no. 00765) and registered at ClinicalTrials.gov (ID: NCT05220371).

A total of 75 healthy male participants aged 18–40 years who had not exercised more than 3 h per week in the preceding weeks, as determined by the IPAQ questionnaire, were randomized after signing a written informal consent. Exclusion criteria for the study included BMI less than 18.5 kg/m^2 or greater than 25 kg/m^2, unstable weight and dietary patterns, symptoms related to physical activity, arterial hypertension, diabetes mellitus, and use of protein supplements within the 6 months before the start of the study. Following American College of Sports Medicine guidelines, individuals with cardiovascular, renal, or metabolic diseases were excluded from the study (ACSM) (27).

Figure 1 depicts the study protocol, which consisted of pre-tests conducted before the training intervention, a 12-week training intervention with a daily SCP or PLA supplementation phase and post-tests conducted after completion of the training intervention. Two weeks before the start of the study (T0), subjects were examined, introduced to the testing procedures, familiarized with the testing equipment, and visited the biomechanics laboratory where all T1 (baseline) and T2 (after 12 weeks) tests were performed. On day 1 of T1, subjects arrived at the laboratory between 7 and 9 am after an overnight fast. A bioelectrical impedance analysis (BIA) was performed to obtain body composition, and a standardized meal (SM) was provided. Participants were required to complete a 5-min warm-up program on a rowing ergometer at moderate intensity one and a half hours after SM. Then, MVC, RFD and CMJ (in this exact order) were performed ("pre"). After the last CMJ, a visual analog scale was used to measure each subject's perception of muscle soreness (MS). After that, 150 drop jumps were performed. Thirty minutes later, MVC, RFD, CMJ and MS were measured again to get "post" results. On days 2 and 3, participants visited the laboratory at the same time as day 1, received their SM, warmed up for 5 min, and performed the same tests (MVC, RFD, CMJ, and MS) to obtain "24 h" and "48 h" values. During T1 and the last 48 h before day 1, participants were asked to abstain from moderate to vigorous physical activity and alcohol to rule out any influence on recovery. From arrival until leaving the biomechanics laboratory on the three consecutive days, they were only allowed to drink water after consuming the standardized meal. After the 12-week training intervention, the same procedure as in T1 was repeated. A room temperature of 20–22°C was ensured on test days.

2.2. Muscle damage-inducing exercise (MDIE)

Part of the pre-test and post-test were 150 drop jumps (6 sets of 25 repetitions, 2 min rest between sets) from a 60 cm high box. Participants were required to land slowly and in a controlled manner at a knee angle of at least 90° to force an adequate eccentric movement and subsequently induce muscle damage, as described elsewhere (28).

2.3. Training intervention

The 12-week training was a concurrent training (CT) session that included both strength and endurance elements, in which the SCP and PLA groups participated to the same extent. Before a 30-min run at a specific heart rate, calculated for each participant using the Karvonen formula (29) (exercise intensity factor between 0.7–0.8; HRmax = 220—age) and monitored by a wrist-mounted fitness tracker, subjects were instructed to perform bodyweight-based lower-body exercises (squats, lunges and calf raises) three times a week, with at least 1 day off between two training days. Weeks 1–4 required 3 sets of 20, weeks 5–8 required 3 sets of 25, and weeks 9–12 required 3 sets of 30 repetitions for each exercise. For endurance training, the individual training intensity factor was increased by 5% after every 4th week. The training sessions took place outdoors on Mondays, Wednesdays and Fridays at 7 am and 6 pm under the supervision of sports scientists at the Institute of Sport Science in Vienna and lasted approximately 1 h. Participants were instructed to exercise regularly, either in the morning or afternoon. A minimum of 26 training sessions were required to successfully complete the study.

2.4. Dietary intake

Subjects were asked to record their food intake on 2 weekdays and one weekend day in week 1 and 12 to gain insight into their dietary habits. Participants noted every meal and beverage they consumed as well as their weight/volume. Nut.s (nutritional.software, dato Denkwerkzeuge, Vienna) was used to calculate total energy intake, fat, protein and carbohydrate.

2.5. Standardized meal (SM)

On each test day, participants consumed an SM consisting of oats and an oat drink 2 h before the first examination. The standardized meal contained 1 g carbohydrate per kg of body weight. At T2, the subjects were also required to take PLA or SCP with their meal for three consecutive days. Participants were prohibited from eating or drinking for 12 h before SM (water excluded on days without BIA measurements). Alcohol consumption was also prohibited 48 h before and during the test days.

2.6. Supplementation

The subjects were randomly assigned to the SCP or PLA group using a research randomizer.[1] Once a day for 12 weeks, they were required to take 15 g of either a placebo (Silicea, metabolically inert) or the test product [mixture of specific collagen peptides (10 g PeptENDURE® + 5 g TENDOFORTE®), Gelita AG, Eberbach, Germany] orally, both similar in taste and consistency (powder), in combination with 0.5 l water. On training days, 7.5 g of the supplement or placebo had to be taken 1 h before and 7.5 g immediately after the training session to ensure high blood SCP levels during and after the training (30–32). On non-exercise days, participants were asked to consume 15 g as a whole at the same time

1 https://www.randomizer.org

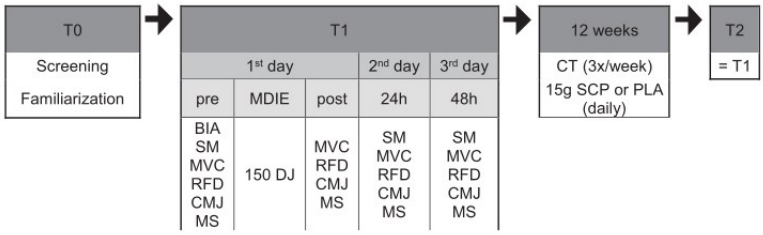


FIGURE 1
Study design in chronological order. BIA, bioelectrical impedance analysis; MVC, maximum voluntary contraction; RFD, rate of force development; CMJ, countermovement jump; MS, muscle soreness; MDIE, muscle damage-inducing exercise; DJ, drop jump; CT, concurrent training; SM, standardized meal.

TABLE 1 Amino acid composition of the specific collagen peptides.

Amino acid	Weight (%)
Hydroxyproline	11.3
Aspartic acid	5.8
Serine	3.2
Glutamic acid	10.1
Glycine	22.1
Histidine	1.2
Arginine	7.8
Threonine	1.8
Alanine	8.5
Proline	12.3
Tyrosine	0.9
Hydroxylysine	1.7
Valine	2.4
Methionine	0.9
Lysine	3.8
Isoleucine	1.3
Leucine	2.7
Phenylalanine	2.1

as on exercise days. At the end of the study (T2), the supplements were taken in combination with the standardized meal before the examination. The amino acid composition of the SCP is listed in Table 1.

2.7. Body composition

BIA (Nutriguard MS, data-input GmbH, Germany) with a frequency of 50 kHz was used to analyze body composition. ECM (extracellular mass), ICM (intracellular mass), FM (fat mass) and FFM (fat-free mass) were recorded. BIA measurements took place on day 1 of T1 and T2 and were the participants' first act after entering the laboratory. After an overnight fast (water was also prohibited), the subjects lay on a mat parallel to the ground. They remained there for 5 min without moving to allow optimal body water distribution. To ensure good conductivity between the skin and electrodes, an electrode spray was applied before attaching two electrodes each on the back of the right hand and the right foot and connecting to the BIA device with cables.

2.8. Maximum voluntary contraction (MVC), rate of force development (RFD) and peak RFD

Subjects began the trials seated (110° hip angle) on a commercial dynamometer (HUMAC NORM Model 770; CSMi, Stoughton, MA, USA) with their hands crossed over the chest, a fastened four-point seatbelt for the upper body, a fastened strap for the leg, and one just above the ankle. The dynamometer position and knee angle (110° for maximum momentum, 180° = full extension) were individually set at baseline. The same position was used for repeated measurements. After the command "3, 2, 1, go," the participants extended their right leg as hard and as far as possible for 3 s for the MVC trials. For the RFD trials, subjects contracted their right leg for a very short time (< 200 ms) with the instruction to generate as much force (i.e., as explosive) as possible as quickly as possible (33). Moreover, the investigators strongly encouraged subjects verbally during the contractions while providing simultaneous visual feedback. As with the CMJ, three valid trials were required for each calculation. The pause between trials was 60 s. The analog signal of joint moment was recorded by the Vicon Nexus A/D card (16 bit) at 1,000 Hz. The resulting data were filtered with a cut-off frequency of 20 Hz based on Winter 2009 (34). A valid MVC trial represents a force held constantly over at least 2 s. The mean of the maximum force (N) from three valid trials was then used for further analysis. When measuring the rate of force development, any countermovement of the tested leg was prohibited and resulted in exclusion from the trial. RFD was calculated as the slope of the force-time curve (Δforce/Δt) over the time interval of 0–50 ms, and force onset was automatically determined when the force signal reached a threshold of ~1.4% of MVC, which is similar to the method described in Aagaard et al. (35). Automatic detection of force onset has already shown high ICC and lower SEM% (36). In addition, the RFD peak (0–100 ms) was also measured, which is a highly reliable parameter (37).

2.9. Countermovement jump (CMJ) and muscle soreness (MS)

After the RFD trials, participants performed three valid CMJs per test point (pre, post, 24 h and 48 h) barefoot. Subjects stood with both feet shoulder-width apart on a single force plate and waited for instructions from study personnel. This was followed by the verbal command "3, 2, 1 and jump," which initiated a countermovement of the participants characterized by a knee angle of 90°. Hands had to be kept on the hips from the beginning until landing. A one-minute break between each trial ensured adequate recovery. After the last CMJ, participants were asked to give feedback on how they felt the pain in their legs during the last jump using a visual analog scale (VAS) with numbers ranging from 0 mm (no pain) to 100 mm (unbearable pain). By applying the impulse-momentum method J = JGRF—JBW = m*vtake-off leading to yflight = ypeak—ytake-off = (vtake-off^2)/2 g, the CMJ height can be calculated (38). The mean value of three valid jumps was used for the further calculations.

2.10. Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics 23 (IBM SPSS Statistics for Windows, Armonk, NY, USA: IBM Corp.). A 95% confidence interval was chosen, and all data are presented as mean ± standard deviation. For the pairwise Student's *t*-test, the normal distribution was checked with the Kolmogorov-Smirnov test to detect changes over time. Before the independent *t*-test, variance homogeneity was also checked with Levene's test. To determine any 3-way interaction effect, a mixed design ANOVA (generalized linear model) was calculated using group (SCP, PLA) as between-subject factor and test time (pre, post, 24 h, 48 h) and study time (T1, T2) as within-subject factors. In addition, the same procedure was performed with test time as a two-subject instead of a four-subject factor to reduce the model (referred to as the "reduced model"), shown in **Supplementary File 1**. The partial eta squared (ηp^2) was reported as the effect size (small effect: $\eta p^2 > 0.01$, medium effect: $\eta p^2 > 0.06$, large effect: $\eta p^2 > 0.14$) (39). To meet the requirements, Mauchly's test of sphericity was also applied. In the event of significance, an ε of < 0.75 led to the Greenhouse-Geisser correction, > 0.75 to that of Huynh Feldt (40). Because the compared groups in the mixed design ANOVA were almost equal in number (largest/smallest < 1.5), indicating robustness of the F-statistics (41), the homogeneity of variance was not checked with Levene's test. All tests were two-sided. *Post hoc* multiple comparisons to illustrate differences between times, groups and within times were performed with the Fisher's least significant difference (LSD) test. For a better illustration of each biomechanical parameter (MVC, RFD, peak RFD, CMJ), the graphs were based on the following procedure: "pre" T1 and T2 were set to 100% for each group. "Post," "24 h" and "48 h" of T1 and T2 were then normalized for "pre" T1 or T2, resulting in a positive or negative% value. The difference (Δ) of each time point was then calculated (e.g., % of post T2 minus% post T1), resulting in an absolute percentage value (="normalized delta"). The mean of all cases per time point and group is shown in **Figures 3–6**. *Post hoc* power analysis was calculated using GPower 3.1.9.7 (Franz Faul, University of Kiel, Germany) and CMJ as parameters. Additional statistical tests of study time × group, study time × test time, group × test time, main effects and numbers without normalized deltas can be found in **Supplementary File 1**.

3. Results

After eligibility testing and positive medical examination, a total of 75 subjects were enrolled, randomized, and allocated to either the SCP (n = 37) or PLA group (n = 38) (**Figure 2**). One participant failed to show up shortly before randomization, so an odd number of subjects were included. Participants lost to follow-up were eliminated for missing too many training sessions (> 10 times). No adverse events or side effects related to SCP or PLA intake were recorded. The per-protocol analysis consisted of 55 participants in total. Participants in the SCP and PLA groups exercised 32.88 and 33.03 times, respectively, during the 12-week intervention (presented as mean). The independent *t*-test revealed no significant difference (p = 0.844) between groups.

3.1. Participant characteristics

Regarding baseline anthropometric data, there were no significant differences in age (p = 0.44), mass (p = 0.35), height (p = 0.51), BMI (p = 0.7), FM (p = 0.27) and FFM (p = 0.53) (**Table 2**).

3.2. Body composition analysis

One participant in the PLA group was excluded due to an error in data recording. There was no significant time, group or interaction effect for the body composition variables. SCP and PLA did not significantly differ (**Table 3**).

3.3. Dietary intake

As illustrated in **Table 4**, no significant time, group or interaction effect was calculated for either the PLA or the SCP group concerning absolute and relative (to body weight) dietary intake.

3.4. Maximum voluntary contraction (MVC)

Due to technical issues on two test days, data were either recorded erroneously or not recorded at all. Therefore, data from 40 participants were used for analysis of MVC and RFD. Mixed ANOVA showed a statistically significant 3-way interaction effect between study time (T1/T2), test time (pre/post/24 h/48 h), and group (SCP/PLA): Greenhouse–Geisser F(1.659, 63.032) = 4.526,

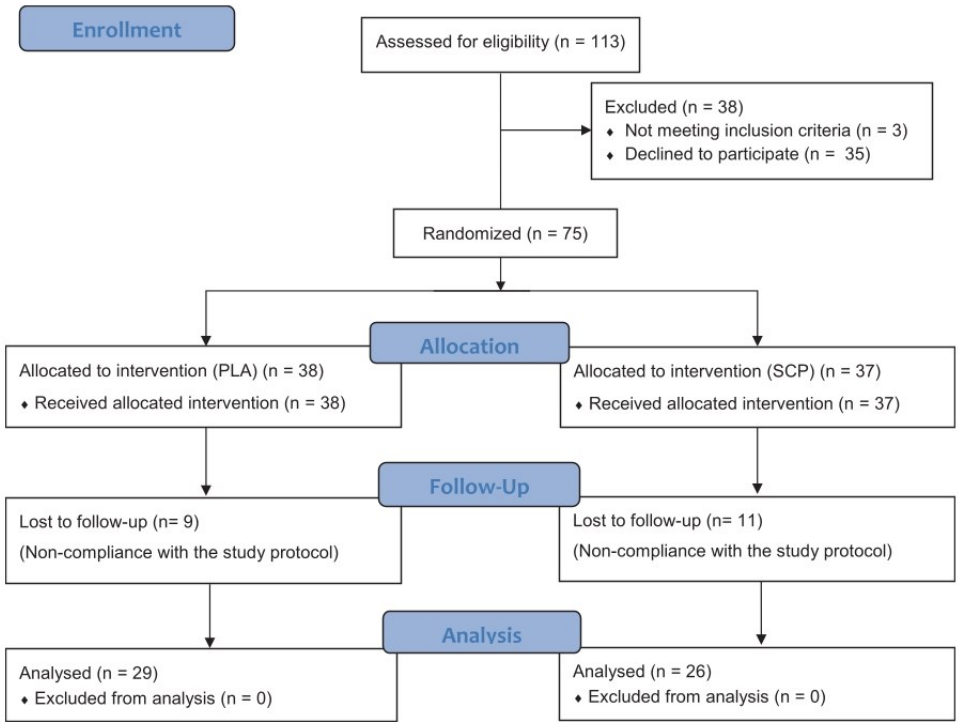


FIGURE 2
CONSORT flow chart.

$p = 0.02$, $\eta p^2 = 0.11$. Normalized deltas were also calculated for each time point for better illustration (Figure 3). Regarding study time (T1 vs. T2) in PLA, a significant difference was found for 24 h ($p = 0.01$) and 48 h ($p < 0.01$), while in MVC, there was no effect in pre ($p = 0.14$) and post ($p = 0.20$) (Table 5). SCP showed significant differences post ($p < 0.01$), 24 h ($p < 0.01$) and 48 h ($p < 0.01$) but not pre ($p = 0.94$). For each time point within the study period (T1 and T2), no differences were found between groups [T1 pre ($p = 0.58$), post ($p = 0.41$), 24 h ($p = 0.12$), 48 h ($p = 0.12$), T2 pre ($p = 0.63$), post ($p = 0.86$), 24 h ($p = 0.72$), 48 h ($p = 0.57$)]. In addition, a comparison of pre with post ($p < 0.01$) and 24 h ($p = 0.01$) showed a significant difference in PLA T1 but not in 48 h ($p = 0.06$). In T2, only post ($p < 0.01$) but not 24 h ($p = 0.25$) and 48 h ($p = 0.69$) values were significantly different from pre. In SCP T1, post ($p < 0.01$), 24 h ($p < 0.01$) and 48 h ($p < 0.01$) showed significant differences compared to pre, while in T2, only post ($p < 0.01$) but not 24 h ($p = 0.22$) and 48 h ($p = 0.68$) was significant.

TABLE 2 Participant characteristics represented as mean ± SD.

Variable	Mean ± SD		Group effect (p-value)
	SCP (n = 26)	PLA (n = 29)	
Age (years)	26.1 ± 5.1	27.2 ± 5.2	0.44
Body mass (kg)	75.0 ± 10.0	77.1 ± 6.3	0.35
Height (m)	1.81 ± 0.1	1.82 ± 0.1	0.51
BMI (kg/m2)	22.8 ± 2	23.3 ± 1.8	0.7
FM (kg)	15.2 ± 5.4	16.3 ± 4	0.27
FFM (kg)	59.8 ± 7.3	61.1 ± 4	0.53

SCP, specific collagen peptides; PLA, placebo; BMI, body mass index; FM, fat mass; FFM, fat-free mass.

3.5. Rate of force development (RFD)

Mixed ANOVA revealed a statistically significant 3-way interaction effect between study time (T1/T2), test time (pre/post/24 h/48 h) and group (SCP/PLA): $F(3, 114) = 8.398$, $p < 0.01$, $\eta p^2 = 0.18$. Normalized deltas for better visualization were also calculated for each time point (Figure 4). Significant differences were also found in RFD in SCP [T1 vs. T2; post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$)], but none in SCP pre ($p = 0.13$) and PLA in pre ($p = 0.13$), post ($p = 0.08$), 24 h ($p = 0.62$) and 48 h ($p = 0.63$) (Table 6). Within the study period, significant differences occurred in PLA T1 pre vs. post ($p < 0.01$), 24 h ($p = 0.05$) but not in 48 h ($p = 0.16$); T2 pre vs. post ($p = 0.01$)

TABLE 3 Body composition analysis represented as mean ± SD.

Variable	SCP (n = 26)		PLA (n = 28)		Interaction effect (p-value)
	T1	T2	T1	T2	
FM (kg)	15.2 ± 5.4	14.2 ± 5.1	16.5 ± 4	15.8 ± 4.1	0.41
FFM (kg)	59.8 ± 7.3	60.7 ± 7.5	60.7 ± 4.3	61.4 ± 4.2	0.56
ECM (kg)	26.8 ± 3.4	27 ± 3.7	27.7 ± 3	27.7 ± 2.6	0.58
BCM (kg)	33 ± 2.4	33.7 ± 4.7	33 ± 2.4	33.7 ± 2.6	0.79

SCP, specific collagen peptides; PLA, placebo; FM, fat mass; FFM, fat-free mass; ECM, extracellular mass; BCM, body cell mass.

TABLE 4 Absolute and relative (rel.) dietary intake without supplement represented as mean ± SD.

Variable	SCP (n = 26)		PLA (n = 29)		Interaction effect (p-value)
	T1	T2	T1	T2	
Energy (kcal)	2,276 ± 663	2,104 ± 429	2,360 ± 640	2,404 ± 513	0.24
Protein (g)	91 ± 31	85 ± 20	93 ± 33	98 ± 23	0.32
Fat (g)	83 ± 25	81 ± 20	89 ± 38	96 ± 30	0.36
Carbohydrate (g)	261 ± 91	234 ± 77	265 ± 58	250 ± 52	0.59
Energy rel. (kcal/kg)	31 ± 10	28.6 ± 6.8	30.2 ± 8.4	31 ± 7.2	0.19
Protein rel. (g/kg)	1.23 ± 0.5	1.15 ± 0.3	1.18 ± 0.4	1.26 ± 0.3	0.26
Fat rel. (g/kg)	1.14 ± 0.4	1.11 ± 0.4	1.14 ± 0.5	1.24 ± 0.4	0.30
Carbohydrate rel. (g/kg)	3.53 ± 1.2	3.17 ± 1	3.4 ± 0.8	3.22 ± 0.8	0.52

SCP, specific collagen peptides; PLA, placebo. Units of relative (rel.) variables are related to kg of body weight.

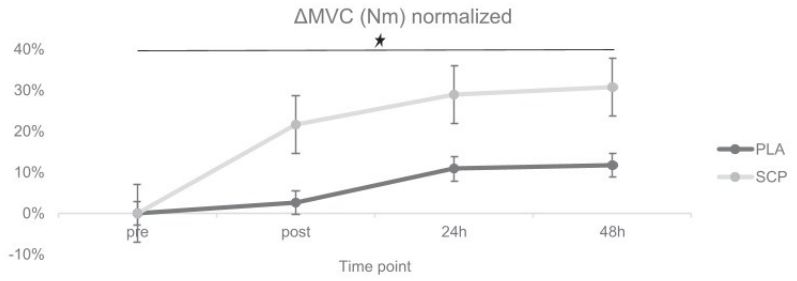


FIGURE 3
ΔMVC (Nm) normalized + standard errors (SE) for each time point demonstrating a significant 3-way interaction effect (*p = 0.02).

TABLE 5 MVC (Nm) represented as mean ± SD.

Group	MVC (Nm)								Interaction effect
	T1				T2				3-way
	Pre	Post	24 h	48 h	Pre	Post	24 h	48 h	
SCP (n = 19)	235 ± 52	170 ± 58a	162 ± 54a	170 ± 59a	236 ± 61	217 ± 59b	230 ± 60*	238 ± 54*	p = 0.02° (ηp^2 = 0.11)
PLA (n = 21)	225 ± 57	186 ± 59a	193 ± 66a	203 ± 73	246 ± 70	213 ± 73*b	237 ± 61*	249 ± 67*	

SCP, specific collagen peptides; PLA, placebo; °, significant 3-way interaction effect. *Post-hoc* tests: *, significant within groups effect (T1 vs. T2); a, significant within groups effect (T1: pre vs. post, 24 h, 48 h); b, significant within groups effect (T2: pre vs. post, 24 h, 48 h).

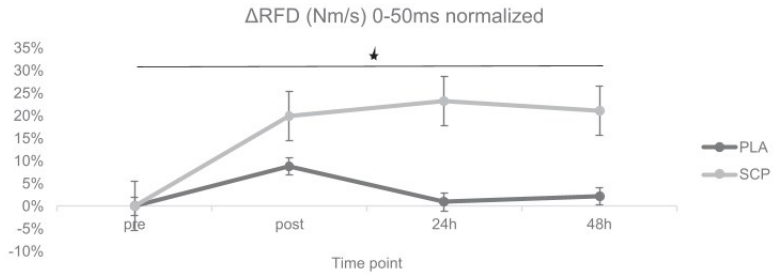


FIGURE 4
ΔRFD (Nm/s) 0–50 ms normalized + SE for each time point demonstrating a significant 3-way interaction effect (*$p < 0.01$).

TABLE 6 RFD (Nm/s) 0–50 ms represented as mean ± SD.

Group	RFD (Nm/s) 0–50 ms								Interaction effect
	T1				T2				3-way
	Pre	Post	24 h	48 h	Pre	Post	24 h	48 h	
SCP ($n = 19$)	1,994 ± 446	1,469 ± 348a	1,513 ± 416a	1,591 ± 388a	1,872 ± 398	1,755 ± 345*b	1,861 ± 459*	1,901 ± 395*	$p < 0.01$° ($\eta p^2 = 0.18$)
PLA ($n = 21$)	1,883 ± 296	1,593 ± 328a	1,764 ± 388a	1,786 ± 396	1,817 ± 288	1,688 ± 318b	1,720 ± 362	1,749 ± 352	

SCP, specific collagen peptides; PLA, placebo; °, significant 3-way interaction effect. *Post-hoc* tests: *, significant within groups effect (T1 vs. T2); a, significant within groups effect (T1: pre vs. post, 24 h, 48 h); b, significant within groups effect (T2: pre vs. post, 24 h, 48 h).

but not in 24 h ($p = 0.09$) and 48 h ($p = 0.36$). In SCP T1, pre vs. post ($p < 0.01$), 24 h ($p < 0.01$) and 48 h ($p < 0.01$) as well as in T2 pre vs. post ($p = 0.04$) were significantly different but not in 24 h ($p = 0.87$) and 48 h ($p = 0.64$) T2. There were no differences between groups [SCP vs. PLA T1 pre ($p = 0.37$), post ($p = 0.26$), 24 h ($p = 0.06$), 48 h ($p = 0.12$); T2 pre ($p = 0.62$), post ($p = 0.53$), 24 h ($p = 0.29$), 48 h ($p = 0.67$)].

3.6. Peak RFD

A statistically significant 3-way interaction effect was identified between study time (T1/T2), test time (pre/post/24 h/48 h) and group (SCP/PLA): F(3, 114) = 6.609, $p < 0.01$, $\eta p^2 = 0.15$. Normalized deltas were also calculated for each time point for better visualization (Figure 5). No statistically significant difference was found between groups at T1 pre [($p = 0.29$), post ($p = 0.27$), 24 h ($p = 0.12$), 48 h ($p = 0.16$)], and T2 [pre ($p = 0.51$), post ($p = 0.63$), 24 h ($p = 0.34$), 48 h ($p = 0.22$)] in peak RFD (Nm/s) 0–100 ms (Table 7). There were differences between groups and study time (T1 vs. T2) in PLA pre ($p = 0.1$) and post ($p = 0.04$) but not in 24 h ($p = 0.87$), 48 h ($p = 0.7$). In SCP, post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$) but not pre ($p = 0.09$) was significant. There were differences within the group in PLA T1 pre vs. post ($p < 0.01$), 24 h ($p = 0.01$) but not vs. 48 h ($p = 0.15$). In T2, pre vs. post ($p = 0.01$), 24 h ($p = 0.13$) but not vs. 48 h ($p = 0.4$). In SCP T1 pre vs. post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$) and T2 pre vs. post ($p = 0.01$) was significantly different, but not in T2 pre vs. 24 h ($p = 0.73$) and 48 h ($p = 0.8$).

3.7. Countermovement jump (CMJ)

Due to illness on two consecutive days, one participant was excluded from the analysis (including the muscle soreness analysis). *Post hoc* power analysis showed a power of 0.959. Mixed ANOVA revealed a statistically significant 3-way interaction effect between study time (T1/T2), test time (pre/post/24 h/48 h) and group (SCP/PLA): Greenhouse–Geisser F(3, 156) = 3.229, $p = 0.046$, $\eta p^2 = 0.06$. Normalized deltas were also calculated for each time point for better illustration (Figure 6). CMJ height differed significantly within groups in PLA T1 pre vs. post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p = 0.02$) but not in T2 [pre vs. post ($p = 0.5$), 24 h ($p = 0.41$), 48 h ($p = 0.15$)] (Table 8). In SCP T1 pre vs. post ($p < 0.01$), 24 h ($p < 0.01$) and 48 h ($p < 0.01$) were significantly different, but none in T2 [pre vs. post ($p = 0.18$), 24 h ($p = 0.74$), 48 h ($p = 0.09$)]. Differences between groups and study time (T1 vs. T2) occurred in PLA pre ($p = 0.04$), post ($p < 0.01$), 24 h ($p < 0.01$) and 48 h ($p < 0.01$), in SCP in post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$) but not in pre ($p = 0.09$). There were no differences between groups in T1 [pre ($p = 0.14$), post ($p = 0.97$), 24 h ($p = 0.9$), 48 h ($p = 0.91$)] or T2 [pre ($p = 0.15$), post ($p = 0.37$), 24 h ($p = 0.21$), 48 h ($p = 0.12$)].

3.8. Muscle soreness

No significant 3-way interaction effect occurred between study time (T1/T2), test time (pre/post/24 h/48 h) and group (SCP/PLA): F(3, 156) = 0.533, $p = 0.66$, $\eta p^2 = 0.01$. Significant differences

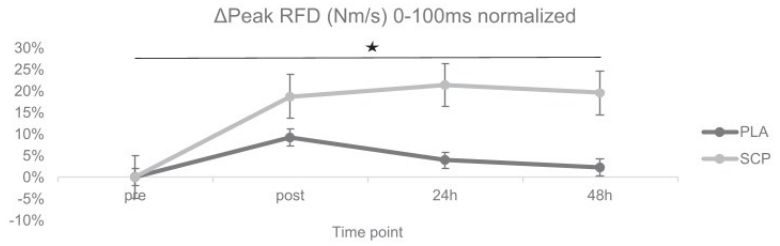


FIGURE 5
ΔPeak RFD (Nm/s) 0–100 ms normalized + SE for each time point demonstrating a significant 3-way interaction effect (*$p < 0.01$).

TABLE 7 Peak RFD (Nm/s) 0–100 ms represented as mean ± SD.

Group	peak RFD (Nm/s) 0–100 ms								Interaction effect
	T1				T2				3-way
	Pre	Post	24 h	48 h	Pre	Post	24 h	48 h	
SCP ($n = 19$)	2,058 ± 422	1,520 ± 336a	1,587 ± 370a	1,667 ± 365a	1,934 ± 402	1,794 ± 363*b	1,912 ± 452*	1,948 ± 379*	$p < 0.01$° ($\eta p^2 = 0.15$)
PLA ($n = 21$)	1,934 ± 297	1,637 ± 332a	1,776 ± 382a	1,834 ± 381	1,862 ± 272*	1,744 ± 296*b	1,789 ± 342	1,808 ± 330	

SCP, specific collagen peptides; PLA, placebo; °, significant 3-way interaction effect. *Post-hoc* tests: *, significant within groups effect (T1 vs. T2); a, significant within groups effect (T1: pre vs. post, 24 h, 48 h); b, significant within groups effect (T2: pre vs. post, 24 h, 48 h).

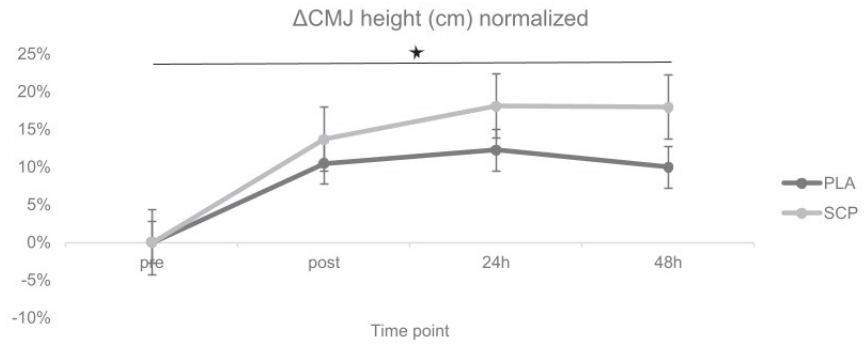


FIGURE 6
ΔCMJ height (cm) normalized + SE for each time point demonstrating a significant 3-way interaction effect (*$p = 0.046$).

between groups in muscle soreness were found in T2 pre ($p = 0.04$) but not in T1 pre ($p = 0.75$), post ($p = 0.31$), 24 h ($p = 0.95$), 48 h ($p = 0.35$), T2 post ($p = 0.33$), 24 h ($p = 0.48$) and 48 h ($p = 0.54$) (Table 9). Within-group differences occurred in PLA T1 pre vs. post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$); T2 pre vs. post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$); SCP T1 pre vs. post ($p < 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$) and T2 pre vs. post ($p = 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$). Within-group-between-study time (T1 vs. T2) was significantly different in PLA pre ($p = 0.02$), post ($p = 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$); in SCP post ($p = 0.01$), 24 h ($p < 0.01$), 48 h ($p < 0.01$) but not in pre ($p = 0.65$).

4. Discussion

The main findings of the study indicate a higher muscular regenerative capacity in subjects supplementing specific collagen peptides (SCP) compared to PLA. This is reflected in significant improvements in the biomechanical parameters maximum voluntary contraction (MVC) and rate of force development (RFD), which recovered significantly faster at each measurement time point (post, 24 and 48 h), and countermovement jump height (CMJ) at 48 h following 150 drop jumps in the SCP group. To the authors' knowledge, this is the first study to examine the effects of daily SCP supplementation combined with

TABLE 8 Countermovement jump height (cm) represented as mean ± SD.

Group	CMJ height (cm)								Interaction effect
	T1				T2				3-way
	Pre	Post	24 h	48 h	Pre	Post	24 h	48 h	
SCP (n = 26)	29.7 ± 6.4	25 ± 6.7a	24.2 ± 7.5a	25 ± 6.6a	30.5 ± 6.1	30 ± 6.1*	30.7 ± 6.7*	31.3 ± 5.8*	p = 0.046° (ηp^2 = 0.06)
PLA (n = 28)	27.2 ± 5.7	24.7 ± 6.9a	24.5 ± 7.5a	25.3 ± 7.5a	28.2 ± 6*	28.4 ± 6.4*	28.5 ± 6.1*	28.8 ± 6.2*	

SCP, specific collagen peptides, PLA, placebo; °, significant 3-way interaction effect. *Post-hoc* tests: *, significant within groups effect (T1 vs. T2); a, significant within groups effect (T1: pre vs. post, 24 h, 48 h).

TABLE 9 Muscle soreness (mm) represented as mean ± SD.

Group	Muscle soreness (mm)								Interaction effect
	T1				T2				3-way
	Pre	Post	24 h	48 h	Pre	Post	24 h	48 h	
SCP (n = 26)	0.2 ± 0.3	2.2 ± 1.8a	3.6 ± 2.1a	3.5 ± 1.8a	0.2 ± 0.4ˆ	1 ± 1*ab	1.3 ± 1*ab	1.1 ± 1.2*ab	p = 0.66
PLA (n = 28)	0.4 ± 0.9	1.8 ± 1.9a	3.6 ± 2.5a	4.1 ± 2.7a	0.1 ± 0.4ˆ*	0.8 ± 0.9*ab	1.1 ± 1*ab	1.2 ± 1*ab	

SCP, specific collagen peptides; PLA, placebo; °, significant 3-way interaction effect. *Post-hoc* tests: *, significant within groups effect (T1 vs. T2); a, significant within groups effect (T1: pre vs. post, 24 h, 48 h); b, significant within groups effect (T2: pre vs. post, 24 h, 48 h).

a 12-week training intervention on parameters associated with muscular recovery.

Previous studies have provided evidence of the acute effects of CP intake and shown an increase in CMJ height after 1 week of supplementation. Prowting et al. (23) and Clifford et al. (24) demonstrated a significant interaction effect (time*group) in favor of CP 24 and 48 h, respectively, after a muscle-damaging exercise bout of 100–150 drop jumps. They concluded that ECM remodeling occurred more rapidly in the passive/elastic tissues in the CP group, resulting in improved force output and power production. Another short-term crossover study found that eight intense training sessions before post-test sessions resulted in a similar CMJ height after 24 h and a more pronounced but non-significant recovery after 48 h in the CP group compared to whey protein supplementation (19). In addition to these acute effects, our results suggest a long-term improvement in reactive strength (CMJ height) following SCP administration one and 2 days after muscle-damaging exercise. Higher stiffness results in more efficient use of elastic energy but is reduced during fatigue states (42). Therefore, it appears that chronic supplementation of SCP resulted in increased stiffness of the force-reinforcing collagenous structures of the lower limbs, reducing their sensitivity to the effects of 150 drop jumps. Dressler et al. (43) demonstrated such adaptations after 6 months of CP supplementation in an athlete population, which led to a slight—although non-significant–increase in ankle stiffness compared to a placebo control.

Maximal strength has been shown to be moderately related to reactive strength (44). In the present study, improvements in MVC recovery were significantly higher in the SCP group than in the placebo group. Despite the lack of statistical significance, the investigation by Oertzen-Hagemann et al. (45) in a group of recreationally active men showed that the additional intake of 15 g CP resulted in greater increases in muscle strength than hypertrophy resistance training (RT) alone. In contrast, previous findings suggest that short-term CP supplementation does not further enhance MVC after muscle-damaging eccentric movements (23, 24) or intense RT (19). Jacinto et al. (46) showed a similar but non-significant behavior of MVC after supplementation (35 g/day) of CP compared to whey protein after 10 weeks of RT. However, the change in muscle thickness, which contributes significantly to maximal strength, was significantly higher in the whey protein group than in the CP group. Since there were no obvious changes in BCM, which is mainly composed of muscle tissue, in both groups, the present study design might lead to a better preservation of neural drive in the CP group.

Neural adaptations, especially motor neuron recruitment speed and discharge rate, strongly affect explosive strength, which is usually determined by measuring RFD in a much shorter time interval (< 200 ms) than MVC (37, 47). The shorter the interval, the smaller the deviation from MVC and thus from muscle size (48) which is why a window of 0–50 ms and 0–100 ms (for peak RFD) was chosen to determine whether SCP affects neural-dependent mechanisms (49). SCP supplementation had a pronounced positive effect on RFD, corroborated by a significant 3-way interaction effect. This is in accordance with Lis et al. (26), who demonstrated a significantly higher percent change in RFD (100 ms) in the CP group compared to PLA after 3 weeks of power training and daily ingestion of 20 g CP. The 50 ms RFD time interval was recently shown to correlate strongly with early (0–50 ms) EMG activity at different knee angles (30°, 45°, 60°, 75°, and 90°), suggesting, in particular, a major contribution of muscle unit discharge rate to early RFD (50). Although there is limited evidence, muscle fiber types may also influence the magnitude of explosive contractions. Type II muscle fibers exhibit a higher rate of tension development and cross-bridge cycling (33). Nonetheless, the high variability in fiber type composition between subjects appears to be problematic

in defining factors that contribute to RFD (51). Therefore, it remains unclear whether SCP also influences fiber type-specific neural responses. Muscle-tendon stiffness may also play a role, as force transmission is enhanced primarily in stiffer junctions. Bojsen-Møller et al. (52) investigated the mechanical properties of the vastus lateralis tendon-aponeurosis complex and its effects on biomechanical parameters. They found a significant correlation between stiffness and RFD_{100ms}, which describes 30% of the variance in RFD in a sample size of 15 subjects. However, caution should be exercised when attributing stiffness to RFD because inter-individual variability is higher than that of trainability (33).

In an athlete population with exercise-related knee pain, CP has been used successfully to significantly reduce pain during activity after 12 weeks and 5 g of CP daily (53, 54). In the present study, participants reported their perceived muscle soreness after all tests were performed at each time point. Activity-related pain would have impacted the outcome of CMJ, a high range-of-motion exercise. However, the muscle soreness data did not differ significantly, suggesting that only subjective pain was recorded, which SCP supplementation may not affect.

Regarding body composition adaptation, a 12-week RT intervention combined with 15 g of CP has already resulted in a significant increase of FFM and a decrease in FM in premenopausal women, elderly, sarcopenic, and untrained middle-aged men (17, 18, 53, 54) (respectively). In contrast, no significant changes in body composition were observed in the SCP and PLA groups in the present study. This may be because CT produced less myofibrillar hypertrophy than RT alone due to the lower volume of RT-based stimuli, which could explain the non-significant difference. In addition, a higher repetition range (20–30, depending on study time) per set and exercise was prescribed, rather than a moderate repetition range of 6–12, recommended for optimizing muscle hypertrophy (55). The present study was primarily concerned with muscle recovery rather than hypertrophy. Therefore, CT rather than RT was used as the training approach. Apart from probably insufficient hypertrophic stimuli, the daily ingestion of 15 g SCP, which is equivalent to $\sim$ 60 kcal according to the Atwater factors (56), also caused no significant changes in body composition. Only a slightly more pronounced (absolute) increase in FFM/decrease in FM was observed in the SCP group (**Table 2**). A 12-week CT combined with 15 g of SCP already significantly increased FFM compared to a non-caloric placebo, at least in experienced recreational female runners (57). In contrast to the present study, the women performed a one-hour endurance run three times a week after RT. The authors mentioned a possible beneficial effect of SCP on peroxisome proliferator co-activator-1a (PGC-1a) and peroxisome proliferator-activated receptor (PPAR), both of which are involved in fat metabolism and stimulation of fatty acid utilization, which has also been demonstrated in mice (58). Perhaps the duration of CT in the current study was too short to induce body compositional adaptations in combination with SCP.

The choice of CT as a training intervention is associated with several physiological adaptations and benefits, most of which are a combination rather than a compromise of those typically resulting from RT or endurance training (ET) alone. A recent systematic review and meta-analysis indicated similar maximal strength and muscle hypertrophy development compared with RT alone (59). Another review confirmed major effects on muscle strength after > 12-week CTs, 30–60 min duration, three sessions per week with high ET intensity and strength before ET (60). However, CT has a negative effect on explosive strength (59), so in the present study, intra-session RT was performed before ET, which could mitigate these impairments. It has been shown that at least 1-RM performance increased significantly when RT was performed in CT before ET (61). Taking this into account, the magnitude of difference over time in RFD in the SCP group is remarkable and supports the possible effect of SCP on explosive strength.

Recovery from exercise-induced muscle damage represents a comprehensive process that occurs in multiple phases and is therefore difficult to assess in its entirety. To date, satellite cells, inflammatory cells, fibro-adipogenic-precursors, vascular tissue, nerve-muscle interaction and the ECM appear to play a central role, not only in the recovery of functionality but also in tissue adaptation to new mechanical stimuli (62). The repeated bout effect (RBE), as a recovery-specific concept and multi-causal physiological mechanism that is not yet fully understood, provides enhanced protection after unaccustomed eccentric exercise, as evidenced by reduced performance decrements, particularly in biomechanical parameters after the second bout. Neural and musculotendinous adaptations, remodeling of the ECM, and inflammation likely regulate RBE (63). Some factors have already been influenced by CP supplementation and therefore represent a promising approach to improving RBE. Since the recorded parameters (CMJ, MVC, RFD) that resulted in significant effects in the present study are biomechanical, improved neural, ECM and/or musculotendinous, complex adaptations are plausible. In the context of ECM remodeling after heavy mechanical loading, the musculotendinous junction (MTJ) displacement recorded in B-mode ultrasound was barely detectable in the biceps brachii 4 weeks after a second bout. The authors suspected that more compliant tendons and muscles were responsible for the apparent change in MTJ displacement (64). As little as 5 g of SCP instead of PLA for 14 weeks significantly increased Achilles tendon cross-sectional area (CSA) but not stiffness and gastrocnemius muscle thickness, once again indicating a potential benefit of SCP in relation to RBE (65). RFD indirectly determined neural adaptations that contributed to the improvement of RBE in the present study and showed a large interaction effect in favor of SCP. No study to date has investigated the effects of CP on neural activation. Therefore, an influence on changes in the activation pattern of motor units as a kind of compensatory strategy, faster recovery of excitation-contraction coupling and/or increased excitability of spinal and motor neurons seem plausible (63, 66, 67). Overall, long-term intake of SCP leads to a significant increase in RBE and thus to improved recovery after exercise-induced muscle damage.

4.1. Limitations

The dropout rate of 28% is higher than in other studies with a 12-week training intervention, which limits the statistical power. Some participants contracted COVID-19 during the study, and participants who had just recovered from a mild infection may not have been able to fully adhere to the study protocol in the following week. This, and the fact that they opted for CT instead of RT, may have led to less adaptation of body composition. The determination of subjective muscle soreness using VAS appeared to be error-prone, with some participants entering values above zero before

the muscle-damaging bout. Moreover, only male participants were included in the study. The results are not generalizable or applicable to females. Therefore, future studies are strongly recommended to include women (68). The results of the present study are also not fully generalizable to other study results using different CP. Finally, meals were not recorded during the test days ("pre" to "48 h") at T1 and T2, which may have influenced the performance outcome.

5. Conclusion

The findings of this study provide evidence for the significant improvement in recovery-related parameters after 12 weeks of supplementation with specific collagen peptides (SCP) in combination with concurrent training, particularly in maximal, explosive, and reactive strength. Interestingly, despite the lack of significant differences in fat-free mass, the improvements in regenerative capacity suggest that adaptations in musculotendinous structures and potential neural adaptations may play a key role in the observed results.

In order to attain a more profound understanding of the underlying mechanisms, future studies should explore the specific effects of SCP on neural activity patterns and molecular adaptations that contribute to enhancing force transmission. Such research will contribute to elucidating the precise molecular pathways and neural mechanisms underlying the observed improvements in strength and recovery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the University of Vienna. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KB: Conceptualization, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft. SS: Formal analysis, Methodology, Supervision, Validation, Visualization, Writing – review and editing. LB: Investigation, Project administration, Writing – review and editing. SO: Formal analysis, Writing – review and editing. AB: Formal analysis, Writing – review and editing. DK: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review and editing.

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Conflict of interest

Collagen peptides and placebo were produced and provided by GELITA GmbH. GELITA GmbH had no influence on the outcome of the study.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1266056/full#supplementary-material

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Reduction in systemic muscle stress markers after exercise-induced muscle damage following concurrent training and supplementation with specific collagen peptides – a randomized controlled trial

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Introduction: Collagen peptide supplementation in conjunction with exercise has been shown to improve structural and functional adaptations of both muscles and the extracellular matrix. This study aimed to explore whether specific collagen peptide (SCP) supplementation combined with a concurrent training intervention can improve muscular stress after exercise-induced muscle damage, verified by reliable blood markers.

Methods: 55 sedentary to moderately active males participating in a concurrent training (CT) intervention (3x/week) for 12 weeks were administered either 15 g of SCP or placebo (PLA) daily. Before (T1) and after the intervention (T2), 150 muscle-damaging drop jumps were performed. Blood samples were collected to measure creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin (MYO) and high-sensitivity C-reactive protein (hsCRP) before, after, and at 2 h, 24 h and 48 h post exercise.

Results: A combination of concurrent training and SCP administration showed statistically significant interaction effects, implying a lower increase in the area under the curve (AUC) of MYO ($p = 0.004$, $\eta p^2 = 0.184$), CK ($p = 0.01$, $\eta p^2 = 0.145$) and LDH ($p = 0.016$, $\eta p^2 = 0.133$) in the SCP group. On closer examination, the absolute mean differences (ΔAUCs) showed statistical significance in MYO ($p = 0.017$, $d = 0.771$), CK ($p = 0.039$, $d = 0.633$) and LDH ($p = 0.016$, $d = 0.764$) by SCP supplementation.

Conclusion: In conclusion, 12weeks of 15g SCP supplementation combined with CT intervention reduced acute markers of exercise-induced muscle damage and improved post-exercise regenerative capacity, as evidenced by the altered post-exercise time course. The current findings indicate that SCP supplementation had a positive effect on the early phase of muscular recovery by either improving the structural integrity of the muscle and extracellular matrix during the training period or by accelerating membrane and cytoskeletal protein repair.

Clinical trial registration: https://www.clinicaltrials.gov/study/NCT05220371?cond=NCT05220371&rank=1, NCT05220371.

KEYWORDS

collagen peptides, muscle damage, concurrent training, recovery, regeneration, creatine kinase, repeated bout effect

1 Introduction

Tissues responsible for proper movement execution (e.g., muscles, tendons) underlie a perpetual process of adaptation in response to specific environmental stimuli. In particular, unaccustomed eccentric exercise is known to induce high mechanical stress, leading to myofibril damage and extracellular matrix (ECM) disruption (1). Many studies have focused on nutritional interventions aimed at improving recovery from locomotor impairments, which are often observed as reductions in functional movement abilities (2–8). In this context, interest in collagen peptide (CP) administration has increased in recent years since regular CP intake has been shown to possibly alleviate joint discomfort, enhance ankle and knee functionality and improve recovery from Achilles tendinopathy, therefore acting as a potential and safe nutritional supplement in sports related injuries (9). Moreover, several short-term studies have reported that CP ingestion is superior to placebo after exercise-induced muscle damage (10–13). Faster restoration of biomechanical parameters such as improved countermovement jump height at 24 h (12) and 48 h (10), improved post-exercise muscle strength at 48 h, and reduced muscle soreness immediately after a muscle-damaging exercise bout (13) have been demonstrated following CP intake in conjunction with exercise. A proteomic analysis illustrated a significantly higher degree of upregulated proteins associated with skeletal muscle fibers, mainly contraction-related changes and structural adaptations to training. This suggests greater changes in collagen-specific proteins after 12 weeks of CP supplementation combined with resistance training (RT) (14). A recent study investigating gene expression patterns following strenuous bouts of knee extensions prior to a single dose of CP showed significant upregulation of the PI3K/Akt and MAPK pathways, both known to be essential for (myofibrillar) protein synthesis (15). The PI3K/Akt pathway may also play a role in connective tissue protein synthesis by mediating TGF-β2 signaling and inducing mRNA expression of COL1A1 & COL1A2 (collagen type 1 genes) in human pigment cells (16). Whether CP supplementation also stimulates connective tissue protein synthesis rates *in vivo* is currently unknown (17), but seems plausible, as increased collagen concentration was measured in human *in vitro*-engineered ligament after a single dose of 15 g CP (18).

Recovery from severe lengthening contractions (e.g., squats or drop jumps) also causes biomarkers in serum blood to peak within the following days. Proteins and metabolic-related enzymes such as creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin (MYO), alanine transaminase (ALT), c-reactive protein (CRP) and interleukin-6 (IL-6) have been considered metabolic indicators of muscle damage and concomitant inflammation, both of which occur after strenuous, mainly eccentric exercise (19). However, there are only two studies without exercise intervention that examined recovery-related blood markers after CP supplementation. Neither found differences between CP and placebo after nine and 42 days of supplementation (10, 11). Therefore, the importance of regular, long-term, and at least moderately intense physical activity in combination with CP ingestion may be of interest for proteomic outcomes related to recovery. Furthermore, the CP administered in this study have only been utilized in two long-term investigations without examining blood related recovery parameters so far (20, 21). Thus, the impact of these particular CPs is also noteworthy.

Based on this assumption, the present study aimed to investigate whether 12 weeks of CP supplementation together with a concurrent training intervention would significantly influence exercise-induced muscular stress. In addition, recovery should be confirmed by specific blood parameters. This could indicate either a reduced acute stress response due to improved functional or structural integrity of the muscles. Or, on the other hand, an optimized muscular regenerative capacity that alleviates both myofibrillar damage and inflammation after exercise-induced muscle damage, or both. Therefore, we hypothesized that the increase of biomarkers in blood after a second bout of muscle-damaging exercise would be lower in subjects taking specific collagen peptides (SCP).

2 Methods

2.1 Study design

The study was a randomized, double-blind, placebo-controlled trial conducted at the University of Vienna, Austria. The study was approved by the Ethics Committee of the University of Vienna (reference no. 00765) and registered at ClinicalTrials.gov (ID: NCT05220371). 75 healthy male participants aged 18–40 years who had not exercised more than 3 hours per week in the preceding months were randomized to the treatment or placebo group after signing a written informed consent. The IPAQ questionnaire quantified individual physical activity levels. Exclusion criteria were a body mass index (BMI) below 18.5 kg/m^2 or above 25 kg/m^2, unstable weight or dietary behavior, physical complaints related to physical activity, and intake of protein supplements within the 6 months before the start of the study. In addition, subjects with renal, cardiovascular or metabolic diseases were excluded in accordance with American College of Sports Medicine (ACSM) guidelines (22).

The study design (Figure 1) comprised pre-tests (T1) before the training intervention, 12 weeks with daily intake of SCP or PLA with concurrent training (3x/week), and study completion with subsequent post-tests (T2) corresponding to the pre-tests. Subjects were screened

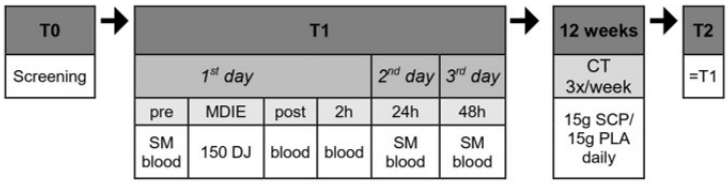


FIGURE 1
Study design in chronological order. Blood, blood collection; MDIE, muscle damage inducing exercise; DJ, drop jump; CT, concurrent training; SM, standardized meal.

2 weeks before T1. Using a research randomizer[1], participants were assigned to the SCP or PLA group after randomization. At baseline, study participants were given a standardized meal (SM) after overnight fasting (7 to 9 a.m.). 1 hour after the SM ("pre"), the first blood samples were collected, followed by the performance of 150 drop jumps. Immediately and 2 hours later, blood samples were collected again ("post," "2 h"). On the second and third days, subjects arrived at the same time as on day 1, received their SM and waited 1 hour until blood was drawn two more times ("24 h," "48 h"). Subjects were required to refrain from physically demanding or unaccustomed exercise and alcohol consumption for 48 h before the trials. Only water was allowed during the test procedures except for the SM.

2.2 Blood samples

Each blood sample was collected venously via venipuncture and filled into 10 mL serum vacutainers. These were centrifuged at 2500 rpm for 15 min, and the resulting supernatant was pipetted into 2 mL Eppendorf tubes and immediately stored in a refrigerator at -32°C. For analysis purposes, the frozen tubes were transported to a local laboratory and analyzed separately. The Beckman Coulter AU5822 (23) was used for detecting creatine kinase (CK), lactate dehydrogenase (LDH) and myoglobin (MYO). High-sensitivity C-reactive protein (hsCRP) was detected "pre" & "24 h" by immunoturbidimetry (Alinity c CRP Vario Reagent Kit, Abbott Laboratories) with a coefficient of variance (CV) of 12.5%. The "24 h" measurement was chosen due to the high likelihood of CRP levels peaking 1 day after intense exercise (24, 25). As some participants were unable to provide blood at the given times, they were excluded from the data analysis.

2.3 Muscle damage-inducing exercise (MDIE)

The MDIE employed consisted of 150 drop jumps from a 60 cm box. Six sets of 25 jumps were prescribed, with a two-minute rest after each set. Participants had to perform the drop jumps slowly and achieve a knee angle of at least 90° to produce sufficient muscle damage, which has been documented elsewhere (26).

1 https://www.randomizer.org

TABLE 1 Amino acids of the specific collagen peptides.

Amino acid	Weight (%)
Glycine	22.1
Proline	12.3
Hydroxyproline	11.3
Glutamic acid	10.1
Alanine	8.5
Arginine	7.8
Aspartic acid	5.8
Lysine	3.8
Serine	3.2
Leucine	2.7
Valine	2.4
Phenylalanine	2.1
Threonine	1.8
Hydroxylysine	1.7
Isoleucine	1.3
Histidine	1.2
Tyrosine	0.9
Methionine	0.9

2.4 Supplementation

15 g of either a placebo (Silicea) or the test product (a mixture of specific collagen peptides [10 g PeptENDURE® + 5 g TENDOFORTE®], Gelita AG, Eberbach, Germany, Table 1) were administered orally daily for 12 weeks in powder form. Both products were mixed with 500 mL of water and drunk. One half of the SCP and PLA packs had to be taken 1 hour before and the second half instantly after the training session. This administration schedule would result in high blood levels of collagen peptides during and after exercise (27–29). On non-training days, the participants consumed 15 g as a whole at the same time as on exercise days.

2.5 Exercise protocol

A concurrent training (CT) intervention was conducted three times weekly for 12 weeks. The first half of the exercise program consisted of

TABLE 2 Concentrations/activity of recovery-related blood biomarkers represented as mean ± SD.

Group	Time									
	T1					T2				
	pre	post	2 h	24 h	48 h	pre	post	2 h	24 h	48 h
CK (U/L)										
PLA (n = 23)	135 ± 64	159 ± 69	182 ± 66	346 ± 197	246 ± 123	142 ± 64	161 ± 66	171 ± 64	214 ± 108	207 ± 140
CP (n = 22)	136 ± 65	174 ± 77	204 ± 88	422 ± 225	506 ± 782	125 ± 66	146 ± 74	156 ± 71	192 ± 133	162 ± 103
LDH (U/L)										
PLA (n = 21)	165 ± 21	181 ± 24	180 ± 24	174 ± 31	175 ± 35	161 ± 30	176 ± 29	176 ± 29	173 ± 27	178 ± 31
CP (n = 22)	181 ± 20	208 ± 21	204 ± 22	195 ± 24	190 ± 22	175 ± 24	185 ± 24	185 ± 24	182 ± 23	188 ± 26
MYO (ng/ml)										
PLA (n = 23)	51 ± 8	89 ± 43	141 ± 108	55 ± 21	49 ± 14	46 ± 9	59 ± 32	77 ± 45	45 ± 13	47 ± 16
CP (n = 21)	48 ± 13	94 ± 50	165 ± 93	62 ± 29	96 ± 120	40 ± 13	46 ± 13	51 ± 14	36 ± 12	35 ± 10
hsCRP (mg/l)										
PLA (n = 21)	0.67 ± 0.4	-	-	1.04 ± 1	-	0.61 ± 0.3	-	-	0.55 ± 0.2	-
CP (n = 19)	1.46 ± 1.4	-	-	2.2 ± 1.8	-	1.21 ± 0.9	-	-	1.23 ± 1	-

lower body weight exercises (squats, lunges and calf raises in chronological order). Starting with three sets of 20 repetitions for each exercise, five repetitions were added after 4 and 8 weeks. For the last 30 min of the CT, subjects ran at a heart rate determined individually according to the Karvonen formula (30) (training intensity factor between 0.7–0.8; HRmax = 220 – age). Pulse watches were provided for each training session to maintain the pace. After 4 and 8 weeks, the individual training intensity factor was increased by 5%. Recovery was ensured by a rest day between two training sessions. Training sessions were conducted outdoors every Monday, Wednesday and Friday at 7 a.m. and 6 p.m. under the supervision of sport scientists at the Institute of Sport Science in Vienna. Participants were encouraged to exercise regularly, either in the morning or afternoon session. Subjects completing fewer than 26 exercise sessions were eliminated from the study.

2.6 Standardized meal (SM)

The SM (oats and an oat drink) was provided at T1 & T2 on every test day, which participants consumed 2 hours before blood collection. The SM contained 1 g of carbohydrate per kg of body weight. In addition, participants were required to ingest 15 g of PLA or SCP with their meal at T2.

2.7 Dietary intake

Using household measurements, participants were required to quantify all the beverages and foods they consumed on two weekdays and one weekend day in weeks 1 and 12. Nut.s (nutritional.software, dato Denkwerkzeuge, Vienna) analyzed the amount of total energy intake, carbohydrates, fats and proteins.

2.8 Statistical analysis

IBM SPSS Statistics 23 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.) was used to perform all statistical analyses. Figures were generated by GraphPad Prism 9. The α-level was set to 5%. Blood marker analysis was based on the data listed in Table 2. These data were used to calculate the area under the curve (AUC) with the linear trapezoidal rule for each blood biomarker (31) and the deltas of these AUCs. Normal distribution was demonstrated with the Kolmogorov–Smirnov test. Dependent t-tests elaborated changes over time. Variance of homogeneity was tested with Levene's test as a prerequisite for the independent t-test. A mixed design ANOVA (generalized linear model with repeated measures) was used for interaction effects, with group (SCP, PLA) as a between-subjects factor and study time (T1, T2) as a within-subjects factor. Partial eta-square (ηp^2) was given as effect size (small effect: $\eta p^2 > 0.01$, medium effect: $\eta p^2 > 0.06$, large effect: $\eta p^2 > 0.14$). The effect size in pairwise t-tests was expressed as Cohen's d (small effect: >0.2, medium effect: >0.5, large effect: >0.8) (32). In the case of a significant Mann–Whitney U test, the effect size was also reported as Cohen's d/ηp^2 [Wolfgang Lenhard & Alexandra (33)]. All tests were two-sided. Due to increased variability in the data sets, outliers were eliminated if they were above the 3rd standard deviation.

3 Results

After meeting the inclusion criteria and a positive medical examination, 75 participants (initially 76, but one subject declined further participation before day 1) were randomized and assigned to the SCP (n = 37) or PLA group (n = 38) (Figure 2). Participants lost to follow-up were excluded for non-compliance (missing more than 10 sessions) with the training protocol. No adverse events or side effects related to SCP or PLA supplementation were observed. 55 subjects successfully completed the study.

3.1 Anthropometrics and dietary intake

Regarding anthropometric parameters (Table 3), no significant differences were observed for age (p = 0.44), mass (p = 0.35), height (p = 0.51) and BMI (p = 0.7). No significant time effect was calculated

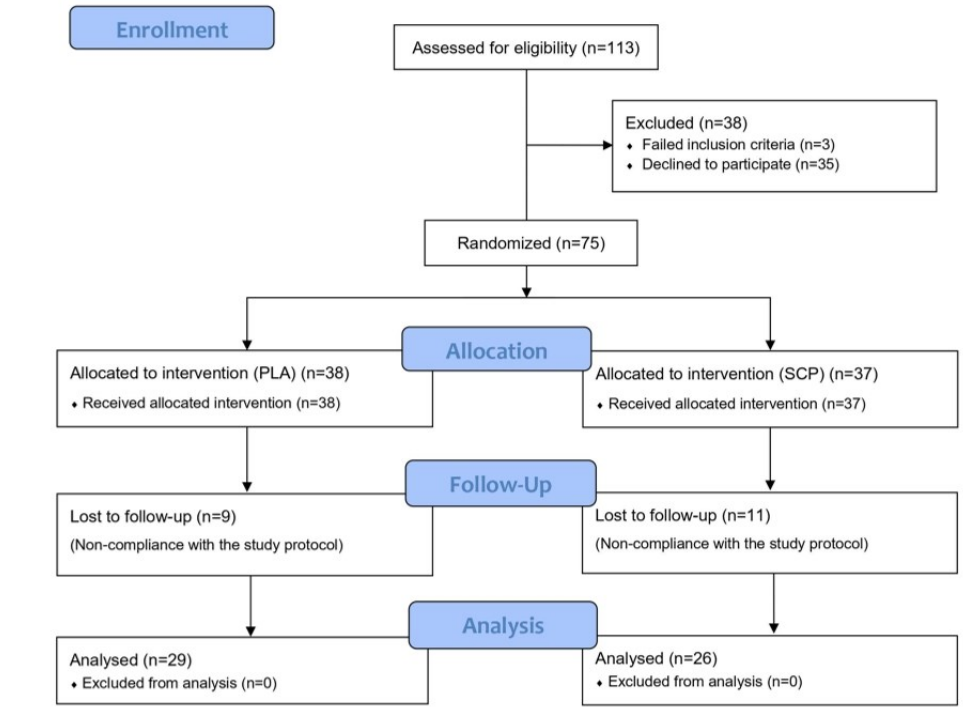


FIGURE 2
CONSORT flow chart.

TABLE 3 Subject characteristics and dietary intake without supplement represented as mean ± SD.

Variable	SCP ($n = 26$)		PLA ($n = 29$)		Mixed ANOVA (p value)
	T1	T2	T1	T2	
Age (years)	26.1 ± 5.1	-	27.2 ± 5.2	-	-
Body mass (kg)	75 ± 10	-	77.1 ± 6.3	-	-
Height (m)	1.81 ± 0.1	-	1.82 ± 0.1	-	-
BMI (kg/m^2)	22.8 ± 2	-	23.3 ± 1.8	-	-
Energy (kcal)	2276 ± 663	2104 ± 429	2360 ± 640	2404 ± 513	0.24
Protein (g)	91 ± 31	85 ± 20	93 ± 33	98 ± 23	0.32
Fat (g)	83 ± 25	81 ± 20	89 ± 38	96 ± 30	0.36
Carbohydrate (g)	261 ± 91	234 ± 77	265 ± 58	250 ± 52	0.59

for PLA [energy ($p = 0.77$), protein ($p = 0.54$), fat ($p = 0.44$), carbohydrate ($p = 0.26$)] and SCP [energy ($p = 0.09$), protein ($p = 0.47$), fat ($p = 0.74$), carbohydrate ($p = 0.06$)] in relation to dietary intake. Similarly, no differences were evident between groups in T1 [energy ($p = 0.71$), protein ($p = 0.72$), fat ($p = 0.55$), carbohydrate ($p = 0.89$)] as well as in T2 [energy ($p = 0.08$), protein ($p = 0.09$), fat ($p = 0.08$), carbohydrate ($p = 0.52$)]. In addition, no interaction effect was observed for energy ($p = 0.24$), protein ($p = 0.32$), fat ($p = 0.36$) and carbohydrate intake ($p = 0.59$).

3.2 Blood biomarkers

Descriptive statistics for CK, LDH, MYO and hsCRP are shown in Table 2, which delineates the data used for further analyses. As some blood samples could not be obtained (entirely) from all subjects who completed the study due to malaise and nausea during the process of blood collection, the number of participants differed slightly between each marker. Individuals who failed to furnish blood at a particular time regarding one

specific parameter were excluded whereas an exclusion was not obligatory for other parameters, if the amount of collected blood was sufficient for their analysis. Therefore, the following numbers of participants have been analyzed: 45 (CK), 43 (LDH), 44 (MYO) and 40 (hsCRP).

3.3 Area under the curve (AUC)

The CK, MYO and hsCRP data were log-transformed due to an initial violation of normal distribution. Significant interaction effects implying a greater reduction in the SCP group were observed in the AUCs of MYO ($p = 0.004$, $\eta p^2 = 0.184$), CK ($p = 0.01$, $\eta p^2 = 0.145$), LDH ($p = 0.016$ $\eta p^2 = 0.133$) but not hsCRP ($p = 0.54$), as shown in Figure 3. Figures 3, 4 demonstrate the data without transformations.

3.4 Delta AUCs

SCP intake resulted in a significantly higher reduction, visualized by ΔAUCs, in MYO ($p = 0.017$, $d = 0.771$/ $\eta p^2 = 0.129$), CK ($p = 0.039$, $d = 0.633$), LDH ($p = 0.016$, $d = 0.764$) but not in hsCRP ($p = 0.915$) (Figure 4). For MYO and hsCRP, the Mann–Whitney U Test was applied as a non-parametric alternative.

4 Discussion

To the best of our knowledge, this is the first study to examine the longer-term impact of a daily intake of specific collagen peptides (SCP) in combination with a 12-week concurrent training (CT) program on muscle stress and recovery-related blood biomarkers. 15 g of SCP significantly decreased the area under the curve (AUC) as well as the ΔAUCs of myoglobin (MYO), creatine kinase (CK) and lactate dehydrogenase (LDH) activity, suggesting improved regenerative capacity within 48 h after muscle damage-inducing bouts of drop jumps.

The results showed better adaptation after eccentric exercise, suggesting a more pronounced repeated bout effect (RBE) after prolonged SCP ingestion. The RBE, a not yet fully understood mechanism that protects tissues involved in muscle lengthening exercise execution from subsequent damage, is thought to be due to neural adaptations, extracellular matrix remodeling, adaptations at the muscle-tendon junction, and inflammation (34).

Severe myofibrillar damage, particularly as it occurs after unaccustomed eccentric exercise bouts, usually results in significant increases in CK, LDH and MYO, which have been extensively used as indirect biomarkers to monitor the extent of acute stress as well as recovery (35). The activity of both cytosolic enzymes (CK, LDH) during a nine (10) and 33-day (13) CP supplementation period was not lower compared to a placebo

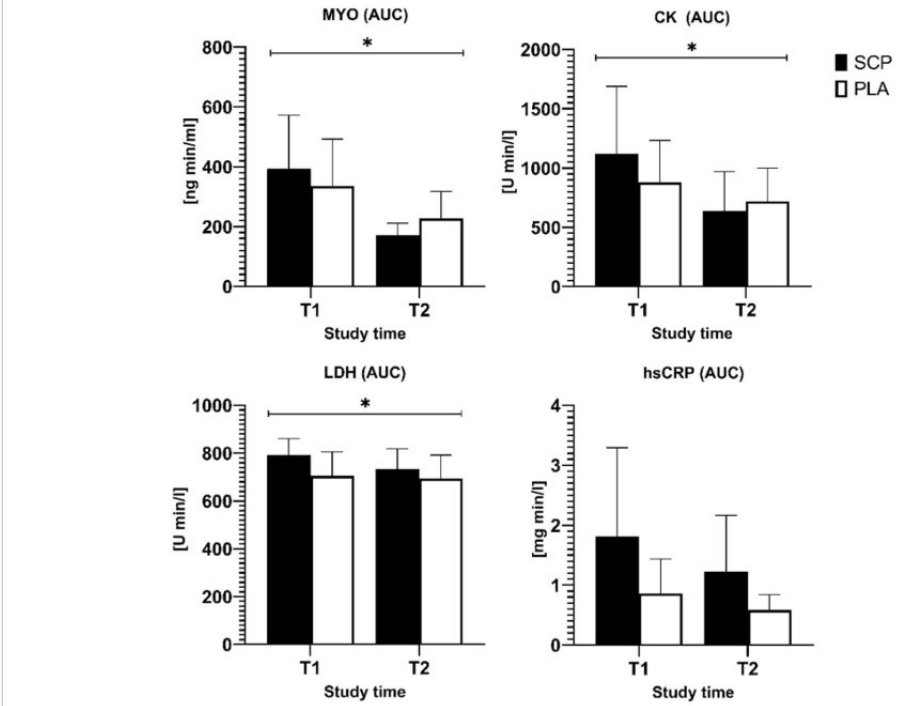


FIGURE 3
Area under the curve of MYO, CK, LDH, and hsCRP. * = significant interaction effect.

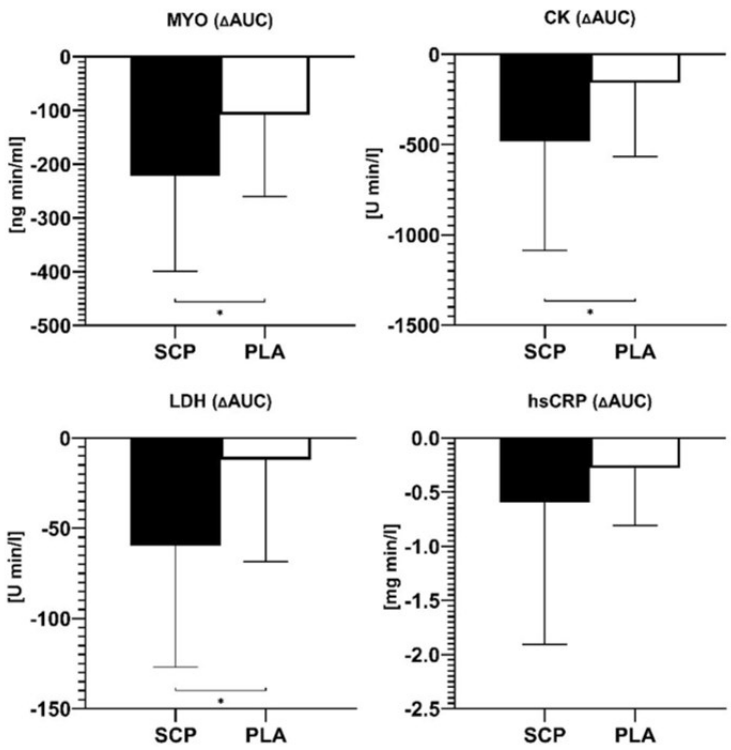


FIGURE 4
Delta AUCs of MYO, CK, LDH, and hsCRP. * = significant group difference.

after muscle-damaging exercise, which could have indicated either reduced acute muscle stress or accelerated reconstruction of damaged tissue. These two studies are the only ones to date that have examined recovery after CP intake by analyzing CK & LDH, albeit in a more short-term manner without prolonged exercise intervention. Therefore, the three-month training intervention in the present study may have improved the remodeling of the force-producing and transmitting tissues (ECM, muscle) with regular SCP administration, reducing the increase in CK & LDH. This hypothesis is supported by the results of a proteomic approach following a 12-week intake of 15 g CP and resistance training (14). The authors identified a 14-fold change in hypoxia-inducible factor alpha 1 (HIF-1α), the alpha subunit of the transcription factor HIF-1, which has shown its contribution to skeletal muscle regeneration in mice (36). HIF-1 exhibits upregulated expression in hypoxic situations, such as exercise, and has been found to play a role in collagen production in rodent tubular epithelial cells (37). Specifically, HIF-1 induces the expression of prolyl and lysyl hydroxylase, two enzymes essential for fiber formation, and promotes proper collagen biogenesis, ECM organization, stiffness, cell adhesion, and motility in human fibroblasts (38). Heat shock protein 90 (HSP90), also upregulated 14-fold in CP in (14), is an HSP chaperone group protein. HSPs are usually overexpressed when exposed to infection, heat-induced stress, hypoxia and thus heat treatment (used as a therapeutic approach), and exercise. Previous studies in mice indicated increased protein levels and markers of muscle hypertrophy/recovery (MHCneo) associated with higher HSP72 levels following downhill running (39). Data from healthy human subjects running downhill for 45 min have shown a negative correlation of ΔMYO and ΔCK along with increasing concentrations of ΔHSP90 (1 h minus baseline) 1 hour after the eccentric endurance bout (40). In addition, Heat shock factor 1 (HSF1), a major transcription factor of HSPs, was also upregulated in CP influenced by HIF-1α in (14), indicating a close interdependence of these proteins (41). It appears that HSPs are involved in protecting cells from damage (42). Still, caution is also warranted when persistently elevated levels are observed, as they indicate chronic inflammation (43). Finally, five and four-fold changes in MAPK and PI3K-Akt pathways have been observed after CP ingestion (14), which are important initiators of signaling cascades leading to protein synthesis. There is little evidence from *in-vitro* experiments that PI3K-Akt & MAPK may also trigger collagen type I gene expression via TGF-β2 activation

(16, 44, 45). TGF-β cytokines generally induce intracellular signaling via specific Smad proteins (Smad2, 3 forming Smad 4 etc.) (46), the latter being responsible for the expression of COL1A2 and COL3A1 (47) which give rise to collagen types I & III, both of which are most abundant in intramuscular connective tissue (48, 49). Given that Smad2, 4 and COL1A1 & COL3A1 mRNA were significantly upregulated 27 (COL1A1) and 30 days after two muscle-damaging bouts of eccentric contractions separated by 27 days (50), the remodeling of muscle ECM, particularly force-bearing proteins such as COL I & III, represents a longer-term process (51). This functional recovery and adaptation process appears to be enhanced by CP supplementation induced by the mechanisms described above.

Based on the CK, LDH & MYO results, the findings suggest increased regenerative capacity throughout ongoing myocellular remodeling. While LDH typically peaks within hours, MYO concentrations and CK activity are highest on the first to third day or, in some cases, even on the fourth day after strenuous exercise, depending on the type of load, muscles used, and individual training history (52–57). Each biomarker leaves its original intracellular location due to cell damage-induced permeability and subsequently enters the bloodstream, where it can be measured in serum/plasma in chronological order (LDH > MYO > CK) (35, 58). In the present study, prolonged SCP intake was shown to ameliorate several cell damage markers and have a beneficial effect on muscle stress response, early-phase muscle recovery, and enhanced RBE. Whether adaptions occurred in surrounding structures such as intramuscular connective tissue and/or muscle fibers *per se* remains unknown.

Regarding inflammatory parameters, the acute-phase protein C-reactive protein (CRP) is a commonly used marker of local and systemic inflammation. In this trial, there was no significant difference between both groups in terms of hsCRP. Considering the measurement time points (baseline and after 24 h), significant CRP changes were already detected after a marathon race (59), a one-hour strenuous endurance run (52), a 90-min eccentric exercise protocol (60) and 100 drop jumps (61). SCP intake had no apparent significant effect on post-exercise inflammatory status in this study; among other factors, the high CV of 12.5% for hsCRP might be responsible for this.

The choice of concurrent training (CT) as the type of training intervention in the present study was based on the following considerations. In general, CT combines the benefits of both RT and ET, at least in untrained to recreational athletes. CT improves physical fitness, does not impair maximal strength and muscle hypertrophy, and is thus consistent with public health guidelines (62). In contrast, CT limits explosive strength gains compared to resistance-only training, offset in the current study by performing RT before ET (63). In agreement with (64), moderate to high ET intensity was prescribed to benefit cardiorespiratory fitness and muscle strength (64). As a recent systematic review mentioned, protein intake (whey, casein, soy, milk) after an acute CT bout increases myofibrillar protein synthesis to a similar extent as after RT (65). Moreover, phosphorylation of the signaling proteins Akt–mTOR-S6K increased significantly immediately after CT and protein intake compared with placebo, indicating enhanced translation of myofibrillar mRNAs (66). CT plus protein also resulted in an immediate post-exercise increase in specific micro RNAs, suggesting a more myogenic rather than oxidative adaptation, and an effect on longer-term CT responses (65, 67). Whether SCP supplementation in combination with CT also leads to the above results remains unknown but may be of interest for future studies.

4.1 Limitations

The present study also has some limitations. Selecting an exercise that also stressed upper limb muscles (e.g., elbow flexors) would have led to more insightful information about the recovery-related setting of blood markers, as upper and lower body muscles have been shown to elicit a slightly different response to MYO & CK (57, 68). In addition, it has recently been suggested that a 30 g dose of milk protein taken immediately after endurance training efficiently repairs and resynthesizes body proteins (65, 69). Whether the same amount of SCP supplementation over a longer period would result in improved recovery of tissues subjected to exercise-induced muscle damage requires future investigation. To date, there is no clinical evidence or recommendations for effective CP dosing to optimize regenerative capacity. In addition, the effects of SCP supplemented in the present study cannot be generalized to other CP products.

4.2 Conclusion

In conclusion, 12 weeks of daily supplementation of 15 g of specific collagen peptides (SCP) in combination with a concurrent training intervention resulted in reduced acute muscle stress response and improvements in early phase recovery, as represented by significantly lower levels of myoglobin (MYO), creatine kinase (CK), and lactate dehydrogenase (LDH) after a second bout of muscle damage-inducing exercise. As this also indicates an enhanced repeated bout effect (RBE), SCPs, together with training, may accelerate repair and reduce cellular damage to the force-producing and transmitting tissues in the first 48 h after eccentric exercise. Future studies should therefore investigate the underlying molecular pathways of myocellular and ECM adaptation after intense muscle-damaging exercises. This may reveal specific target mechanisms affected by SCP intake that could be of significant practical importance in reducing rehabilitation and recovery time and improving RBE.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of the University of Vienna (reference no. 00765). The studies were conducted in accordance with the local legislation and institutional

requirements. The participants provided their written informed consent to participate in this study.

Author contributions

KB: Writing – original draft, Writing – review & editing. SS: Writing – review & editing. LB: Writing – review & editing. SO: Writing – review & editing. AB: Writing – review & editing. DK: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW

Impact of Collagen Peptide Supplementation in Combination with Long-Term Physical Training on Strength, Musculotendinous Remodeling, Functional Recovery, and Body Composition in Healthy Adults: A Systematic Review with Meta-analysis

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Abstract

Introduction Over the past decade, collagen peptide (CP) supplements have received considerable attention in sports nutrition research. These supplements have shown promising results in improving personal health, enhancing athletic performance, and preventing injuries in some but not all studies.

Objective A systematic review and meta-analysis of randomized controlled trials (RCTs) has been conducted to investigate the effects of long-term daily collagen peptide (CP) supplementation on strength, musculotendinous adaptation, functional recovery, and body composition in healthy adults, both with and without concurrent exercise interventions over several weeks.

Methods The PRISMA with PERSiST guidelines were followed for this systematic literature review, which was conducted in December 2023 using PubMed, Scopus, CINAHL, and SPORTDiscus databases. Eligible studies included healthy, normal to overweight adults over 17 years of age who engaged in exercise and daily collagen peptide (CP) supplementation for a minimum of 8 weeks (except one 3-week trial only included for maximal strength). Studies examining recovery-related outcomes were also eligible if they included a 1-week supplementation period without exercise. Methodological study quality was assessed using the PEDro scale. A random-effects model with standardized mean differences (SMD) of change scores was chosen to calculate overall effect sizes.

Results Nineteen studies comprising 768 participants were included in both the systematic review and meta-analysis. Results indicate statistically significant effects in favor of long-term CP intake regarding fat-free mass (FFM) (SMD 0.48, $p < 0.01$), tendon morphology (SMD 0.67, $p < 0.01$), muscle architecture (SMD 0.39, $p < 0.01$), maximal strength (SMD 0.19, $p < 0.01$), and 48 h recovery in reactive strength following exercise-induced muscle damage (SMD 0.43, $p = 0.045$). The GRADE approach revealed a moderate certainty of evidence for body composition, a very low certainty for tendon morphology and mechanical properties, and a low certainty for the remaining.

Conclusion This systematic review and meta-analysis represents the first comprehensive investigation into the effects of long-term CP supplementation combined with regular physical training on various aspects of musculoskeletal health in adults. The findings indicate significant, though of low to moderate certainty, evidence of improvements in fat-free mass (FFM), tendon morphology, muscle mass, maximal strength, and recovery in reactive strength following exercise-induced muscle damage. However, further research is required to fully understand the mechanisms underlying these effects, particularly regarding tendon mechanical properties and short-term adaptations to collagen peptide (CP) intake without exercise, as observed in recovery outcomes. Overall, CP supplementation appears promising as a beneficial adjunct to physical training for enhancing musculoskeletal performance in adults.

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Key Points

Long-term intake of collagen peptide supplementation in conjunction with resistance or concurrent training seems to offer advantages for active individuals and athletes aiming to improve fat-free mass, maximal strength, tendon morphology, and reactive strength recovery.

Enhancing the tendinous cross-sectional area through collagen peptides has the potential to act preventively against sports-related tendon injuries.

Based on current data, achieving the desired adaptations seems to require a daily intake of 15 g of CP for at least 8 weeks; however, more research is needed on this topic.

1 Introduction

Recently, global sales of food supplements, in particular of collagen peptides (CP), have surged tremendously, reaching a market value of 599 million USD in 2020 [1]. This trend underscores an urgent need for comprehensive scientific research to evaluate the current evidence on (CP) usage, particularly in the domains of sports nutrition and rehabilitation. The need for systematic scientific assessment is further emphasized by the increasing number of publications on this topic.

At present, 40 mammalian collagen genes have been identified, encoding 29 specific collagen types, with types I and III being the most abundant in muscles [2, 3]. Myofibrillar proteins are crucial for generating force, while collagenous tissue, such as intramuscular connective tissue, plays a significant role in force transmission, underscoring its importance in overall force output [4]. Given the repetitive amino acid sequence (Gly–X–Y) and the resulting abundance of glycine, proline (Pro), and hydroxyproline (Hyp) in collagen proteins, the current rationale for potential effects of CP supplementation may hinge on the integration of these amino acids into musculotendinous tissues derived from the ingested CP [5, 6]. Shaw and colleagues [7] have already reported a significant uptake of these particular amino acids within 2 h after administration of 15 g gelatin and 6 min of rope-skipping compared with placebo in human serum. Furthermore, both collagen content and concentration increased significantly in engineered human ligaments after the ingestion of 15 g [7]. Postprandial absorption trials without exercise demonstrated peak concentrations of certain collagen di- and tripeptides in human blood [8] and mouse skin [9] after 60 and 15 min, respectively. Furthermore, it has been shown that absorption of enzymatically hydrolyzed collagen protein is higher than that of nonhydrolyzed collagen [10]. Prolonged daily CP supplementation resulted in higher plasma Hyp peptide levels 1 and 2 weeks following the initial CP intake in young and older adults (20–60 years) [11]. Mouse skin fibroblasts [12] and cartilage tissue [13] also exhibited elevated levels of Pro–Hyp and Pro, respectively, after acute collagen administration, indicating an overall enhanced uptake of specific CPs. Notably, as gelatin and CPs are sometimes used interchangeably, water-soluble gelatin is derived from heat-exposed and hydrolyzed collagen [14], which can be further processed into collagen peptides through enzymatic breakdown [15]. Compared with gelatin (~ 100 kDa) [16], CPs generally have a lower molecular weight (~ 0.5–3 kDa) [17], exhibit serum peptidase resistance, and have been observed to be absorbed in larger portions than tripeptides [18]. This enhances the tissue incorporation of consumed CPs and potentially elicits biological effects as bioactive peptides.

Protein supplementation in combination with resistance training (RT) is known to increase lean body mass and reduce fat mass as demonstrated by a recent meta-analysis that primarily included studies utilizing protein sources rich in essential amino acids (EAA) (e.g., whey protein) [19]. However, CPs are generally considered low in EAAs and are often categorized as low-quality protein [20]. Despite this, CP supplementation has shown significant impacts on fat-free mass (FFM) in various populations. For example, in older adults, daily ingestion of 40 g of CP, whey protein, or whey protein combined with RT over the course of a year did not result in differences in FFM [21]. A 12-week RT program with middle-aged, untrained men supplementing 15 g of CP daily led to a significant increase in FFM and loss in fat mass (FM) compared with a placebo [22]. Similarly, premenopausal women participating in a 12-week concurrent training (CT) regimen experienced significant gains in FFM when supplemented with 15 g of CP [23]. Additionally, a significant loss of FM and augmentation in FFM has been reported in elderly sarcopenic men [24]. A combination of (predominantly) resistance training (RT) and timely CP supplementation appears to provide the strongest stimulus for upregulating anabolic pathways such as phosphatidylinositol 3-kinase–protein kinase B (PI3k-Akt), mitogen-activated protein kinase (MAPK), and the mechanistic target of rapamycin (mTOR) to induce myofibrillar hypertrophy and collagen synthesis [25]. Some trials also included vitamin C supplementation alongside CPs, as there is limited evidence demonstrating increased collagen type I synthesis and reduced oxidative stress [26, 27].

Numerous exercise intervention studies have investigated the potential benefits of regular CP intake on musculotendinous adaptation processes; however, the results were not always consistent. For instance, a study involving 15 weeks

of lower-body resistance training (RT) found a significantly greater extent of myofibrillar hypertrophy in the vastus medialis, as measured by magnetic resonance imaging, with daily intake of 15 g of CP compared with a placebo. However, this effect was not observed in the overall exercised muscles (quadriceps + hamstrings + gluteus maximus) [28]. In another study assessing both muscle cross-sectional area (CSA) and thickness, CP supplementation led to similar increases in vastus lateralis following a 10-week RT intervention compared with a mixture of whey protein and creatine [29]. Long-term experiments investigating tendinous remodeling have also demonstrated equivocal outcomes. For example, intake of 15 g of CP over more than 3 months, combined with RT, did not produce superior gains in patellar tendon CSA or stiffness [30]. In contrast, 14 weeks of 5 g of CP alongside a similar exercise intervention showed significantly greater CSA in both Achilles and patellar tendons [31, 32].

As tendons play a pivotal role in transferring contractile force, the influence of CP intake on biomechanical parameters such as maximal voluntary contraction (MVC) and reactive strength, usually determined by countermovement jump (CMJ) height, has also been investigated in healthy adults. A study employing a 12-week strength training protocol combined with daily administration of 15 g of CP demonstrated similar gains in leg strength (leg press) and significantly higher handgrip strength compared with a placebo [33]. The rate of force development (RFD) in collegiate athletes has also been significantly improved following 3 weeks of RT and 20 g of CP [34]. Moreover, in a study focused on functional recovery, the restoration of baseline CMJ height following exercise-induced muscle damage was significantly enhanced after 12 weeks of CT and 15 g CP [35]. Muscle soreness, serving as an indicator of muscle damage, has also been extensively investigated in the context of CP supplementation but yielded contradictory results [36–38] likely influenced by varying study designs, especially differences in the duration of supplemental interventions.

So far, just one systematic review without meta-analyses examined possible effects of CP supplementation on body composition, collagen synthesis, and recovery from joint injury and exercise [39]. The authors concluded that 15 g daily appeared to be effective for improving body composition as well as enhancing collagen synthesis in respective tissues; in addition, they found that CP intake might also moderately improve muscle recovery [39]. Since then, several studies focusing on CP supplementation in the field of sports nutrition have been published. The fact that study populations, administered dosages, outcome parameters, and durations contain at least some heterogeneity, a meta-analysis has been conducted, aiming for a comprehensive statistical investigation of the aforementioned aspects. Both comprehensive quantitative and qualitative research performed in this review aims to reveal whether CP intake linked with exercise, in particular RT, is associated with improvements in sports performance-related injury-preventive conditions. Therefore, we analyzed the extent of remodeling in body composition, muscles, and tendons and possible adaptation in strength and biomechanical recovery-related outcomes.

2 Methods

The present systematic review and meta-analysis was prospectively registered at Open Science Framework (Registration DOI: https://doi.org/https://doi.org/10.17605/OSF.IO/WCF4Y) and written on the basis of PERSiST (implementing Prisma in Exercise, Rehabilitation, Sport medicine and SporTs science) guidelines [40].

2.1 Eligibility Criteria

Studies investigating healthy female and male adults (> 17 years of age) with a lower and upper body mass index (BMI) limit of 18.5–31 kg/m^2 (a BMI of 31 due to one study being also suitable for this meta-analysis [22]) and no preceded periods of ankle or joint pain were included. Both low and professional training status (sedentary to five trainings a week) of subjects were suitable for inclusion. Participants had to ingest a daily dose of CP (as treatment group) for at least 3 weeks, and the control group received a calorie- or non-calorie-matched placebo regardless of manufacturer and added vitamin C. As one article appeared suitable with a supplementation regimen of three times a week, this one was also considered for meta-analysis [41]. All included studies exclusively administered CPs instead of gelatin. To be also eligible, no energy restriction (e.g., phases of hypoenergetic states) during the intervention period was allowed. Moreover, only studies with an accompanying training intervention, either endurance, resistance, or concurrent training, under normobaric conditions of at least two times a week and a minimum of 3 weeks were included (except recovery-related studies, where duration of the supplementation phase was constituted to a minimum of 1 week without obligatory training intervention). Manuscripts had to be English- or German-written randomized controlled trials (RCT). Animal, in vitro, and ex vivo studies were excluded. Studies adding creatine or caffeine as fortification were also excluded.

2.2 Information Sources and Search Strategy

Literature search was carried out in December 2023 in PubMed, Scopus, CINAHL, and SPORTDiscus (last both

via EBSCO) without restrictions regarding publication year, language, and authors—only any kind of review was excluded via filters. A search string was applied in all databases: "collagen AND (peptide OR peptides OR supplement OR supplementation OR hydrolysate) AND (recovery OR muscle strength OR body composition OR architecture OR tendon OR muscle OR performance) [AND (only added in Scopus)] NOT (disease OR skin OR osteoporosis OR osteoarthritis)." "Clinical Trials" and "Randomized Control Trials" filters in PubMed and "Article title, Abstract, Keywords" instead of "All fields" in Scopus were additionally used for further specification. References of included studies as well as Google Scholar were also screened (forward and backward search) and, in case of eligibility, handpicked. Gray literature was not actively sought but considered if eligibility criteria were met. The results of the search process are illustrated in Fig. 1.

2.3 Selection and Data Collection Process

After initial search string query in all databases, literature screening was performed by two independent reviewers (A.M.M., K.B.) with the free online Rayyan tool (https://www.rayyan.ai/). If discrepancies of in-/exclusion of articles occurred following the screening of title, abstract and full-text, the two reviewers resolved them by consensus. If not, a third reviewer (S.S.) made the final decision. Data of included studies were collected by K.B. from articles directly or authors upon request (in most cases).

2.4 Data Extraction

Number of participants, study design, age, training status, dose, type of training intervention, and placebo as well as outcome parameters were extracted from included studies and can be located in Table 1. If data could not be found

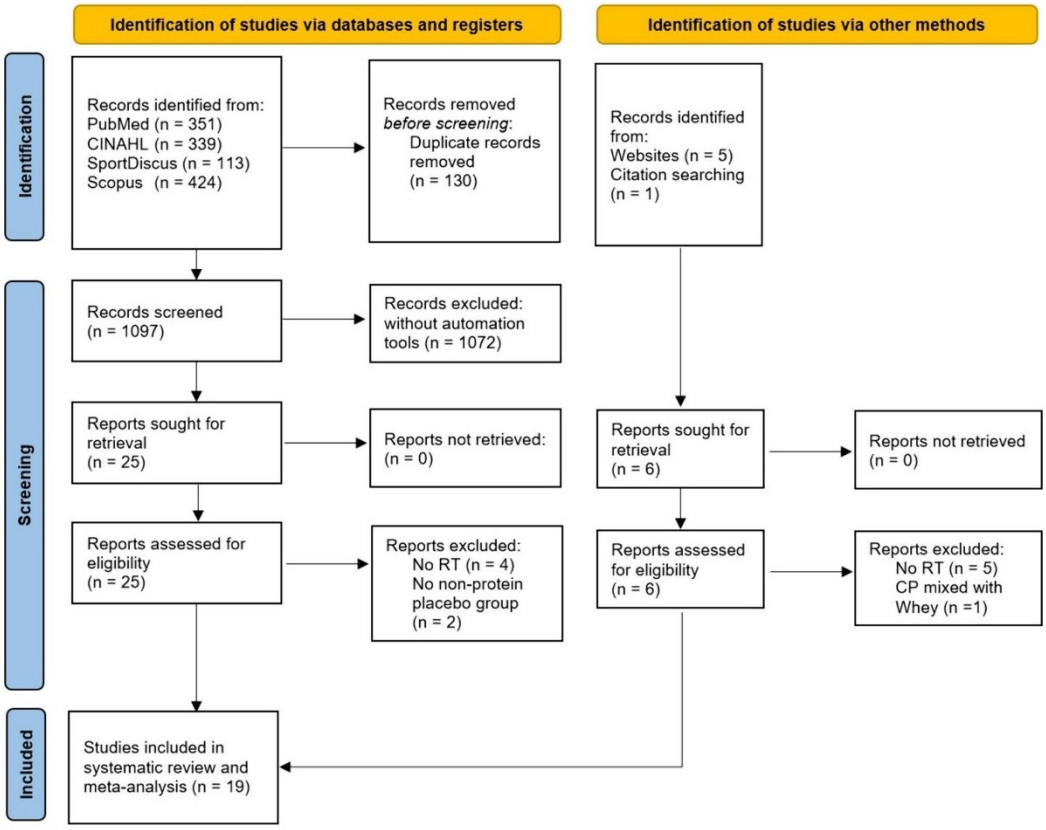


Fig. 1 PRISMA flow chart

inside articles, corresponding authors were contacted via email for request. For one particular study [38], WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/) was used to extract mean ± standard deviation (SD) from graphs. If only standard error (SE) instead of SD could be found, SD was calculated by $SD = SE \times \sqrt{N}$ [42]. In regard to the meta-analysis, the following individual parameters per study were chosen for each item. Fat-free mass: whole body fat-free mass obtained via dual-energy X-ray absorptiometry (DXA) or bioelectrical impedance analysis (BIA). Muscular adaptation: volume (cm^3) of quadriceps (and also rectus femoris, vastus intermedius, lateralis and medialis alone), gluteus maximus and hamstrings; CSA (cm^2) of the thighs; thickness (cm) of medial gastrocnemius, vastus lateralis, intermedius and rectus femoris. Tendinous remodeling: (a) tendon functional properties: Young's modulus (GPa) of patellar tendon (PT) and stiffness (N/mm) of PT and Achilles tendon (AT), (b) tendon morphological properties: CSA (mm^2) of PT and AT. Body composition: fat-free mass (FFM) (kg). Strength: MVC (Nm, N) of knee extension and flexion and plantar flexion using a dynamometer or 90° leg press; one-repetition maximum (1RM) (kg) of squat, deadlift, bench press, bent-over row (all with barbells), knee extension on a dynamometer and handgrip with a hand dynamometer; maximal isometric squat performance (%) and squat jump (N), both assessed via force plates. Recovery: MVC (Nm) of knee extensions using a dynamometer, CMJ height (cm) with force plates or optical instruments, muscle soreness (MS) (mm) by means of a visual analog scale—values from post, 24 h and 48 h after exercise-induced muscle damage. Since only two studies investigated CMJ height post exercise, 24 h and 48 h without "post" has been included in this meta-analysis.

2.5 Risk-of-Bias Assessment

To evaluate RCT's risk of bias, the Physiotherapy Evidence Database (PEDro) scale was used as an equivalent alternative to the Cochrane risk of bias tool [43]. Scores and their respective rank are stated as follows: < 4 are considered "poor," 4–5 are considered "fair," 6–8 are considered "good," and 9–10 are considered "excellent" [44]. A.M.M. and K.B. carried out the methodological assessment of the final included studies. A third reviewer (S.S.) resolved any disagreements if necessary. Studies below a "fair" score (< 6) were excluded from the analysis. Additionally, funnel plots visualized and Egger's regression test calculated risk of publication bias in "maximal strength." All other parameters were not suitable for publication bias assessment by means of funnel plots since a minimum of ten studies are required. However, funnel plots for all parameters are provided for additional information (Fig. S1 and Fig. S2 in supplementary file).

2.6 Meta-analysis

Meta-analysis was conducted in Jamovi 2.4.11 (open statistical software) with the "MAJOR" package 1.2.4 (W. Kyle Hamilton) by using the standardized mean difference (SMD) of change scores (post–pre values, resulting in Δmean and ΔSD). Therefore, studies only providing pre/post data without delta (only one study [37]) had to be recalculated, as mixing up change scores and pre/post data is intended only for mean differences (MD) [42]. The recalculation was performed using a correlation coefficient ("corr") of 0.5 [45] and 0.7 [46] to get the ΔSD with the following formula: $\Delta SD = \sqrt{(SD_{pre}{}^2 + SD_{post}{}^2 - 2 \times corr \times SD_{pre} \times SD_{post})}$ [47]. In case the results of using 0.5 and 0.7 were similar, 0.5 was chosen for meta-analysis. A random-effects model was applied within all variables, and effect sizes are constituted as SMD with Δmean ± ΔSD and a 95% confidence interval since some studies varied slightly in outcome scales (e.g., cm^2 and cm^3 for muscle architectural purposes). The model estimators DerSimonian–Laird, restricted maximum likelihood, and Paul–Mandel were applied. Since there were no significant differences in outcomes when utilizing them, the restricted maximum-likelihood estimator was employed throughout all analyses. I^2 (25%, 50%, and 75% are considered low, moderate, and high variance, respectively [47]), the Cochran's Q statistic ($p < 0.05$ indicating studies do not share a common effect size [48]), and τ^2 as an indicator of between-study variance were calculated as measures of heterogeneity. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to evaluate certainty of evidence [49]. Since all included studies depicted RCTs, grading started at "high" certainty. Outcomes could be downgraded to "very low" certainty due to risk of bias, inconsistency, indirectness, imprecision, and publication bias. Upgrading evidence was not possible in RCTs.

3 Results

After duplicate removal, 1097 studies were manually screened, of which 25 appeared to meet eligibility criteria. Due to some limitations such as no training intervention and an insufficient control group design, 19 studies, all of them being RCTs published in 2015–2023, were included in the systematic review (Fig. 1). Six records identified from websites and citation searches were excluded due to aforementioned reasons.

In total, 768 subjects, of which 613 were male and 155 were female, participated in the included studies. Of these 768, 122 completed five studies that collected only recovery-related data. The age of participants ranged from ~ 17 to 65, with one study exclusively including elderly above 65 years

Table 1 Characteristics of included studies in the systematic review and meta-analysis

Study	*n*	Design	Subjects	Training status	Dose	Intervention	Placebo	Outcome (Δ)
Balshaw et al. [30]	39	RCT	18- to 40-year-old males	Low–moderate PA	15 g	15 weeks RT	Maltodextrin	Young's modulus (GPa) CP = 0.12 ± 0.22, PLA = 0.17 ± 0.23 PT CSA (mm^2) CP = 1 ± 3.1, PLA = 1.5 ± 3.2 PT stiffness (N/mm) CP = 242 ± 415, PLA = 322 ± 397
Balshaw et al. [28]	39	RCT	18- to 40-year-old males	Low–moderate PA	15 g	15 weeks RT	Maltodextrin	Muscle volume (cm^3) Quads + hamstrings + gluteus maximus: CP = 524.5 ± 226.3, PLA = 418.9 ± 125.4 Quads only CP = 271.7 ± 130.6, PLA = 205.1 ± 90.6 MVC (Nm) KE: CP = 46.3 ± 15.6, PLA = 49.7 ± 22.8 KF: CP = 21.9 ± 12.5, PLA = 20.1 ± 13.6 KE 1RM (kg) CP = 14.8 ± 5.6, PLA = 14.2 ± 6
Centner et al. [50]	30	RCT	60.1 ± 7.6-year-old males	1 h/week	15 g	8 weeks BFR	Silicea	Muscle CSA (cm^2) CP = 9.56 ± 4.27, PLA = 8.91 ± 4.28 Leg strength (N) CP = 108.7 ± 315, PLA = 51.2 ± 139.4
Jendricke et al. [33]	77	RCT	18–50-year-old females	Untrained	15 g	12 weeks RT	Silicea	FFM (kg) CP = 1 ± 0.89, PLA = 0.38 ± 0.87 Leg strength (N) CP = 250.8 ± 147.4, PLA = 233.6 ± 182.9 Handgrip strength (kg) CP = 2.7 ± 2.1, PLA = 1.3 ± 2.4
Jendricke et al. [23]	59	RCT	18–40-year-old females	Running 2 ×/week for 60 min	15 g	12 weeks CT	Silicea	FFM (kg) CP = 0.8 ± 0.9, PLA = 0.3 ± 1 Leg strength (kg) CP = 5 ± 5.2, PLA = 6.6 ± 6.3

Table 1 (continued)

Study	*n*	Design	Subjects	Training status	Dose	Intervention	Placebo	Outcome (Δ)
Jerger et al. [32]	31	RCT	18–40-year-old males	2 h/week	5 g	14 weeks RT	Maltodextrin	PT CSA (mm^2) CP = 10.94 ± 6.61, PLA = 6.25 ± 5.87 60% and 70% of PT length CP = 10.73 ± 7.76, PLA = 4.19 ± 6.04 PT stiffness (N/mm) CP = 286.9 ± 394.9, PLA = 292.8 ± 565.7 Leg strength (kg) CP = 54.49 ± 40.27, PLA = 68.05 ± 42.73 KE 1RM (kg) CP = 26.94 ± 11.05, PLA = 27.1 ± 20.58
Jerger et al. [31]	40	RCT	18–40-year-old males	1 h/week	5 g	14 weeks RT	Maltodextrin	Muscle thickness (cm) CP = 0.16 ± 0.12, PLA = 0.06 ± 0.06 AT CSA (mm^2) CP = 7.4 ± 3.51, PLA = 3.13 ± 2.82 AT stiffness (N/mm) CP = 64.23 ± 107.77, PLA = 139.62 ± 154.3 MVC (Nm) CP = 21.75 ± 19.23, PLA = 25.63 ± 20.42
Jerger et al. [89]	32	RCT	18–40-year-old males	Running 2×/week for 60 min	15 g	12 weeks CT	Silicea	FFM (kg) CP = 0.2 ± 1.2, PLA = 0.5 ± 1.3
Kirmse et al. [51]	57	RCT	24 ± 3-year-old males	Moderate RT	15 g	12 weeks RT	Silicea	Muscle thickness (mm) VL: CP = 2.66 ± 1.62, PLA = 1.99 ± 1.93 RF: CP = 0.64 ± 1.94, PLA = 0.49 ± 1.82 INT: CP = 1.9 ± 2.19, PLA = 1.03 ± 2.64 FFM (kg) CP = 2.05 ± 1.84, PLA = 0.63 ± 1.31 1RM (kg) SQ: CP = 20.6 ± 11.44, PLA = 14.82 ± 9.22 DL: CP = 21.35 ± 13.98, PLA = 19.4 ± 10.99 BP: CP = 12.85 ± 4.46, PLA = 11.8 ± 5.68 MVC (Nm) CP = 25.24 ± 32.94, PLA = 22.56 ± 24.88

Table 1 (continued)

Study	*n*	Design	Subjects	Training status	Dose	Intervention	Placebo	Outcome (Δ)
Lee et al. [41]	17	RCT	< 21-year-old females	Elite soccer	30 g (+0.5 g vitamin C)	10 weeks soccer-specific + bodyweight strength and plyometrics	Maltodextrin + fructose + 0.5 g vitamin C	Young's modulus (GPa) CP = 0.20 ± 0.15, PLA = 0.07 ± 0.13 Muscle thickness (mm) CP = 0.44 ± 2.15, PLA = 0.18 ± 1.31 PT CSA (mm^2) CP = 0.15 ± 0.24, PLA = 0.01 ± 0.08 PT stiffness (N/mm) CP = 373 ± 222, PLA = 123 ± 255 MVC (Nm) KE: CP = 16.8 ± 28.1, PLA = 2.2 ± 31.3 KF: CP = −4.4 ± 14.3, PLA = −3.3 ± 12.4
Lis et al. 2022 [34]	48	RCT	18–25-year-old males	Collegiate rugby players	20 g (+50 mg vitamin C)	3 weeks rugby + RT	Maltodextrin	MIS (%) CP = 7.81 ± 2.6, PLA = 7.09 ± 2.8 SJ (N) CP = 64.9 ± 399.8, PLA = 7.6 ± 270.7
Oertzen-Hagemann et al. [90]	25	RCT	24 ± 2.6-year-old males	Moderate RT	15 g	12 weeks RT	Silicea	FFM (kg) CP = 2.56 ± 2.22, PLA = 0.7 ± 1.14 1 RM (kg) SQ: CP = 26.5 ± 13.2, PLA = 17.6 ± 9.9 DL: CP = 24.5 ± 19.1, PLA = 15.9 ± 13.9 BP: CP = 14 ± 6.6, PLA = 9.8 ± 5.5 R: CP = 13.3 ± 8.7, PLA = 6.1 ± 5.8 MVC (Nm) CP = 29.4 ± 22.9, PLA = 14.5 ± 14.3
Zdzieblik et al. [22]	97	RCT	30–60-year-old males	1 h/week	15 g	12 weeks RT	Silicea	FFM (kg) CP = 3.4 ± 2.5, PLA = 1.8 ± 2.1 Leg strength (N) CP = 163 ± 189, PLA = 100 ± 154 Handgrip strength (kg) CP = 1.2 ± 3.7, PLA = 1.8 ± 5.7
Zdzieblik et al. [24]	53	RCT	> 65-year-old males (with sarcopenia)	< 1 h/week	15 g	12 weeks RT	Silicea	FFM (kg) CP = 4.2 ± 5.1, PLA = 2.9 ± 5.2 MVC (Nm) CP = 16.12 ± 5.1, PLA = 7.38 ± 5.2

Table 1 (continued)

Study	*n*	Design	Subjects	Training status	Dose	Intervention	Placebo	Outcome (Δ)
Recovery-related studies								
Bischof et al. [35]	55	RCT	18–40-year-old males	3 h/week	15 g	12 weeks CT	Silicea	FFM (kg) CP = 0.98 ± 1.89, PLA = 0.67 ± 2.07 Muscle soreness (mm) Post: CP = − 1.13 ± 2.0, PLA = − 0.99 ± 1.65 24 h: CP = − 2.28 ± 1.97, PLA = − 2.52 ± 2.17 48 h: CP = − 2.45 ± 1.78, PLA = − 2.98 ± 2.51 CMJ (cm) Post: CP = 4.89 ± 3.96, PLA = 3.56 ± 2.69 24 h: CP = 6.47 ± 6.15, PLA = 4.01 ± 3.99 48 h: CP = 6.3 ± 5.32, PLA = 3.48 ± 3.83 MVC (Nm) Post: CP = 46.8 ± 56.4, PLA = 27.5 ± 50.1 24 h: CP = 67.3 ± 67.01, PLA = 43.9 ± 63.2 48 h: CP = 68.1 ± 62.8, PLA = 45.9 ± 57.2
Kuwaba et al. [36]	20	RCT	40–65-year-old males	No current regular exercise	10 g	4 weeks no TR	Dextrin	Muscle soreness (mm) Post: CP = 27.36 ± 24.42, PLA = 39.3 ± 26.8 24 h: CP = 26.75 ± 23.77, PLA = 26.7 ± 19.6 48 h: CP = 22.08 ± 20.7, PLA = 20.8 ± 20.7
Prowting et al. [71]	15	RCT	18–35-year-old males	Resistance trained (3 ×/week)	15 g	12 days RT	Cornstarch	(all SDs calculated with corr = 0.5) Muscle soreness (mm) 24 h: CP = 35 ± 18, PLA = 29 ± 26 48 h: CP = 30.6 ± 22.4, PLA = 13.1 ± 16.2 CMJ (cm) 24 h: CP = − 1.26 ± 8.8, PLA = − 4.99 ± 7.7 48 h: CP = − 1.84 ± 8.8, PLA = − 0.21 ± 8.6 MVC (Nm) LQ 24 h: CP = − 6.1 ± 27, PLA = − 8.3 ± 30 LQ 48 h: CP = − 4.1 ± 27, PLA = − 1.8 ± 31 RQ 24 h: CP = − 2.3 ± 21, PLA = − 12.5 ± 29 RQ 48 h: CP = − 2.6 ± 21, PLA = − 1 ± 30

Table 1 (continued)

Study	*n*	Design	Subjects	Training status	Dose	Intervention	Placebo	Outcome (Δ)
Clifford et al. [37]	24	RCT	~24-year-old males	2 days/week	20 g (+80 mg vitamin C)	9 days no TR	Maltodextrin	(all SDs calculated with corr 0.5) Muscle soreness (mm) Post: CP = 67.8 ± 40.2, PLA = 82.5 ± 52.2 24 h: CP = 94.5 ± 39.9, PLA = 123.6 ± 31.2 48 h: CP = 78.3 ± 41.2, PLA = 110.3 ± 32 CMJ (cm) Post: CP = − 3.7 ± 8.2, PLA = − 3.1 ± 7.2 24 h: CP = − 4.1 ± 8.6, PLA = − 6.3 ± 7.1 48 h: CP = − 3 ± 8.9, PLA = − 6.6 ± 7.6 MVC (Nm) Post: CP = − 115 ± 91, PLA = − 105 ± 140 24 h: CP = − 93 ± 114, PLA = − 115 ± 157 48 h: CP = − 72 ± 112, PLA = − 98 ± 155
Lopez et al. [38]	8	RCT	18–55 years old, both sexes	Recreationally active	3 g	6 weeks no TR	Cellulose	(all SDs calculated with corr 0.5) Muscle soreness (mm) Post: CP = 1.6 ± 3.9, PLA = 0.6 ± 3.1 48 h: CP = 7.7 ± 12.7, PLA = 3.1 ± 6.8

RCT randomized controlled trial, *PA* physical activity, *w* week, *d* day, *RT* resistance training, *CT* concurrent training, *TR* training, *CP* collagen peptide group, *PLA* placebo group, *PT* patellar tendon, *AT* Achilles tendon, *CSA* cross-sectional area, *MVC* maximal voluntary contraction, *KE* knee extension, *KF* knee flexion, *1RM* one-repetition maximum, *FFM* fat-free mass, *VL* vastus lateralis, *RF* rectus femoris, *INT* vastus intermedius, *SQ* squat, *DL* deadlift, *BP* bench press, *R* bent-over row, *MIS* Maximal isometric squat, *SJ* Squat jump, *LQ* left quadriceps, *RQ* right quadriceps, *post/24 h/48 h* change scores referring to values obtained preceding muscle damage induced exercise

[24]. CP and PLA were administered daily (except in [41] where three times a week was planned), amounting between 3 and 30 g with 15 g (12 studies) and with a single serving each day being the most common supplementation pattern. Moreover, vitamin C (50, 80, and 500 mg) was also added in three experiments, since vitamin C may increase collagen synthesis [27]. Two studies recruited highly trained athletes, whereas others included subjects ranging from untrained to exercising 3 h per week or three times per week prior to study commencements. Non-recovery-associated studies typically lasted at least 3 weeks, while the majority of studies performed interventions lasting 12–15 weeks. Out of five recovery-related investigations, three did not prescribe any training at all; they rather supplemented 9–28 days and indirectly measured (via MVC, CMJ, and MS) muscle damage immediately before and several times following muscle damage inducing exercises. The other two included a 3-week RT and a 12-week CT. In the last one, participants had to perform tests both before and after the training intervention [35], which was therefore a "unique" approach in this context. Regarding the type of training intervention, one study held a blood-flow-restriction RT, three a concurrent training (body-weight exercises combined with running), and the rest either a body-weight-specific or barbell- and machine-based RT. Maltodextrin, fructose, dextrin, cornstarch, and cellulose were administered as isocaloric placebos, whereas silicea (nine studies) constituted a non-calorie-matched placebo. Table 2 illustrates the risk-of-bias assessment via PEDro scores. None of the included studies reached a total score below "good" (6–8), which implicates low risk of bias overall. Most of the studies failed to satisfy criteria 8 and 9 (11 and 13 studies, respectively), due to missing intention-to-treat analyses and a dropout rate higher than 15%.

Fat-free mass Eight studies including 418 subjects investigating fat-free mass changes following long-term CP supplementation (Fig. 2) revealed a significant pooled effect size (ES) of 0.48 [$p < 0.01$, confidence interval (CI) 0.22–0.74]. Two studies used DXA [22, 24], and six remaining studies

Table 2 Risk-of-bias assessment expressed as PEDro scores

Study	1	2	3	4	5	6	7	8	9	10	11	Total
Balshaw et al. [30]	1	1	1	1	1	1	1	0	0	1	1	**8**
Balshaw et al. [28]	1	1	1	1	1	1	1	0	0	1	1	**8**
Bischof et al. [35]	1	1	1	1	1	1	1	0	0	1	1	**8**
Centner et al. [50]	1	1	1	1	1	1	1	0	0	1	1	**8**
Clifford et al. [37]	1	1	1	1	1	1	1	1	1	1	1	**10**
Jendricke et al. [33]	1	1	1	1	1	1	1	1	1	1	1	**10**
Jendricke et al. [23]	1	1	1	0	1	1	1	0	0	1	1	**7**
Jerger et al. [32]	1	1	1	1	1	1	1	0	0	1	1	**8**
Jerger et al. [31]	1	1	1	1	1	1	1	0	0	1	1	**8**
Jerger et al. [89]	1	1	1	1	1	1	1	0	0	1	1	**8**
Kirmse et al. [51]	1	1	1	1	1	1	1	1	0	1	1	**9**
Kuwaba et al. [36]	1	1	1	1	1	1	1	0	0	1	1	**8**
Lee et al. [41]	1	1	0	1	1	1	0	0	0	1	1	**6**
Lis et al. [34]	1	1	1	1	1	1	1	1	0	1	1	**9**
Lopez et al. [38]	1	1	1	1	1	1	1	1	1	0	1	**9**
Oertzen-Hagemann et al. [90]	1	1	1	1	1	1	1	1	1	1	1	**10**
Prowting et al. [71]	1	1	1	1	1	1	1	1	1	1	1	**10**
Zdzieblik et al. [22]	1	1	1	1	1	1	1	0	1	1	1	**9**
Zdzieblik et al. [24]	1	1	1	1	1	1	1	1	0	1	1	**9**

Total scores are in bold. Also include definitions: 1 = criterion satisfied, 0 = not satisfied. Rating: < 4 "poor", 4–5 "fair", 6–8 "good", 9–10 "excellent" (the higher, the better). Criterions 1-11 are listed. Cristerion 1 is mandatory, but is usually not included in the calculation

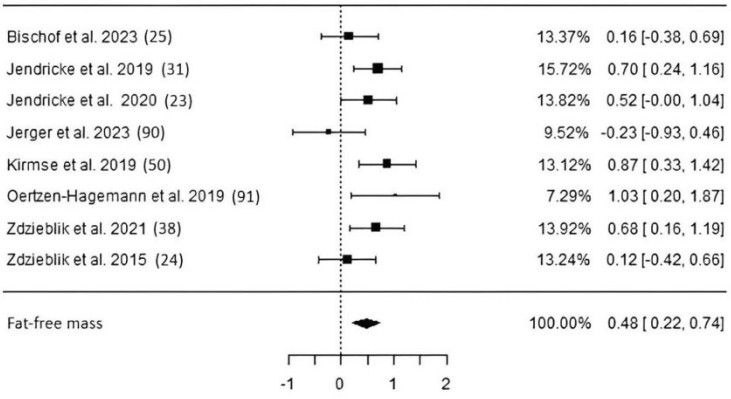


Fig. 2 Studies investigating fat-free mass with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals

chose BIA to obtain body composition. Low heterogeneity along with low risk of publication bias has been detected (Table 3).

Tendon morphology Four studies including 114 subjects obtained data on patellar and Achilles tendon cross-sectional area by using either magnetic resonance imaging (MRI) [30, 32] or ultrasound (US) [31, 41]. A significant pooled ES of 0.67 ($p = 0.01$, CI 0.16–1.19) with moderate heterogeneity and low risk of bias was found (Table 3, Fig. 3). It is noteworthy that when Jerger et al. [32] was excluded, where 60% and 70% of the PT CSA was also measured and data are provided in the article, the random-effects model would not reach statistical significance ($p = 0.058$).

Tendon mechanical properties Four studies, including 111 participants from which data of Young's modulus and stiffness of either PT or AT by means of sonography were acquired, resulted in a nonsignificant pooled ES of 0.05 ($p = 0.82$, CI − 0.38 to 0.48) as shown in Fig. 4.

Table 3 Estimated SMD, heterogeneity statistics and publication bias assessment (by means of Egger's regression test)

Parameter	Estimate	*p*	τ^2	I^2 (%)	*Q*	*p* (*Q*)	Egger's regression test (*p*)
Fat-free mass	0.48	**< 0.001**	0.054	40	12.237	0.093	0.924
Tendon morphology	0.672	**0.011**	0.19	55.52	9.217	0.056	0.141
Tendon mechanical properties	0.049	0.822	0.126	45.37	9.446	0.093	0.011
Muscle architecture	0.387	**< 0.001**	0	0	4.985	0.662	0.593
Maximal strength	0.192	**0.002**	0	0	25.701	0.48	0.615
Recovery maximal strength post	0.145	0.474	0	0	0.791	0.673	0.486
Recovery maximal strength 24 h	0.26	0.144	0	0	0.622	0.961	1
Recovery maximal strength 48 h	0.306	0.087	0	0	2,114	0.715	0.211
Recovery reactive strength 24 h	0.414	0.051	0	0	0.166	0.920	0.806
With corr 0.7	0.452	**0.033**	0	0	0.105	0.949	0.988
Recovery reactive strength 48 h	0.427	**0.045**	0	0	1.75	0.417	0.232
Recovery muscle soreness post	−0.208	0.256	0	0	1.217	0.749	0.791
Recovery muscle soreness 24 h	−0.049	0.785	0	0	3.447	0.328	0.489
Recovery muscle soreness 48 h	0.093	0.703	0.117	40.79	6.78	0.148	0.715

Statistically significant results are in bold

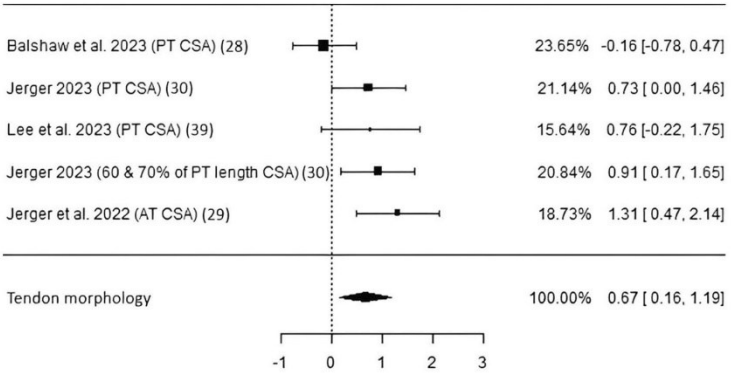


Fig. 3 Studies investigating tendon morphology with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals. *PT* patellar tendon, *AT* Achilles tendon, *CSA* cross-sectional area

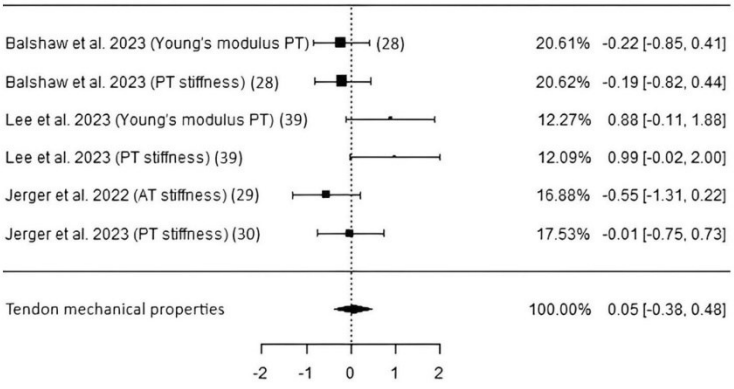


Fig. 4 Studies investigating tendon mechanical properties with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals. *PT* patellar tendon, *AT* Achilles tendon

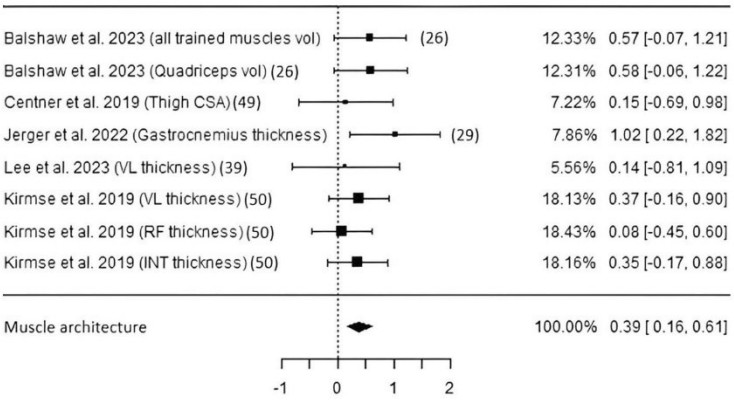


Fig. 5 Studies investigating muscle architecture with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals. *Vol* volume, *CSA* cross-sectional area, *VL* vastus lateralis, *RF* rectus femoris, *INT* vastus intermedius

Heterogeneity appeared to be low, and Egger's regression test hinted potential publication bias ($p = 0.01$; Table 3). Moreover, Ref. [41], having a PEDro score of merely 6 and therefore being the only study with the lowest score within the systematic review and meta-analysis, apparently causes overall heterogeneity.

Muscle architecture Five studies including 161 subjects investigated muscular adaptations using either MRI [28, 50] or sonography [31, 41, 51]. As seen in Fig. 5, CP intake led to a statistically significant pooled estimate (ES 0.39, $p < 0.01$, CI 0.16–0.61). Risk of bias as well as heterogeneity were of low potential/values (Table 3 and Fig. S1).

Maximal strength Eleven studies including about 511 subjects (depending on selected parameters) resulted in a significantly higher maximal strength following CP supplementation (ES 0.19, $p < 0.01$, CI 0.07–0.31; Fig. 6) compared with placebo. Maximal strength was measured by means of a dynamometer, leg press, multijoint resistance exercise (weights), or a force plate. Heterogeneity and risk of bias appeared to be low (Table 3, Fig. S1). The exclusion

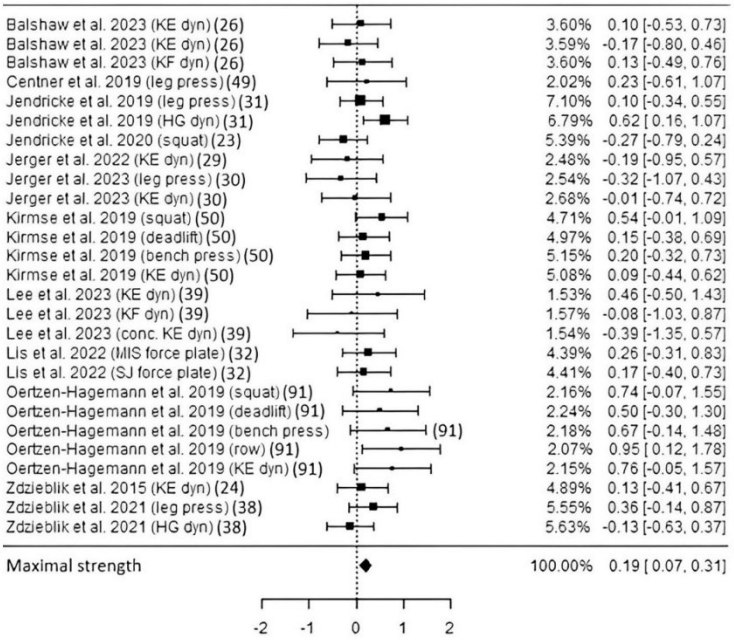


Fig. 6 Studies investigating maximal strength with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals. The first two values differed in units and instructions, but both derived from using a dynamometer. *KE* knee extension, *KF* knee flexion, *dyn* dynamometer used, *HG* handgrip, *conc.* concentric, *MIS* maximal isometric squat, *SJ* squat jump

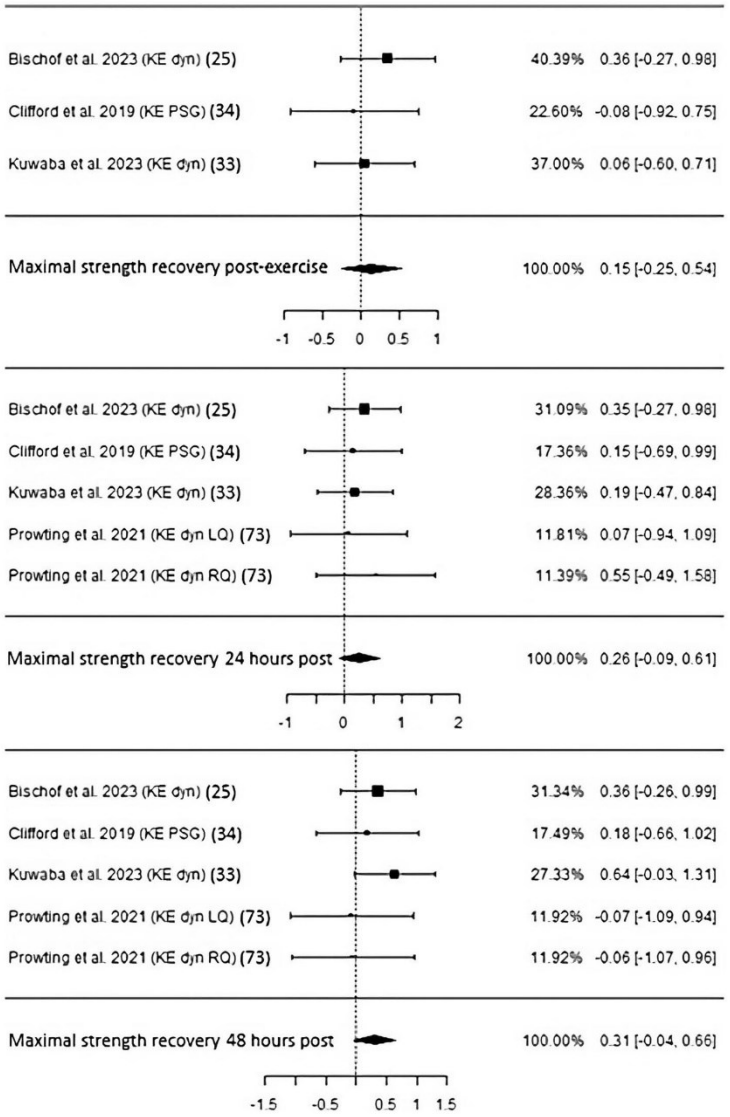


Fig. 7 Studies investigating maximal strength recovery immediately post, 24 h, and 48 h following exercise-induced muscle damage with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals. Studies investigating with individual weights, SMD and confidence intervals. PSG, portable strain gauge

of the 3-week trial by Lis et al. 2022 [34] led to similar gains in maximal strength (ES 0.19, $p < 0.01$, CI 0.06–0.32), indicating long-term adaptation elicited by CP administration.

Recovery of maximal and reactive strength and muscle soreness For immediate regeneration of maximal strength after exercise-induced muscle damage, three studies with 98 participants were not able to reach statistical significance (ES 0.15, $p = 0.47$, CI − 0.25 to 0.54; Fig. 7). Both heterogeneity and publication bias were of low values (Table 3).

Twenty-four hours following muscle damaging exercise(s) that either comprised several sets of muscle lengthening drop jumps [35, 37], squats [36] or an upper-body resistance training [38] did not lead to statistical significance (ES 0.26, $p = 0.14$, CI − 0.09 to 0.61). Four studies including 113 participants overall presented low risk of bias and heterogeneity. The same number of experiments and subjects also failed to reach significance (ES 0.31, $p = 0.09$, CI − 0.04 to 0.66). Heterogeneity and the potential of risk of bias stayed low.

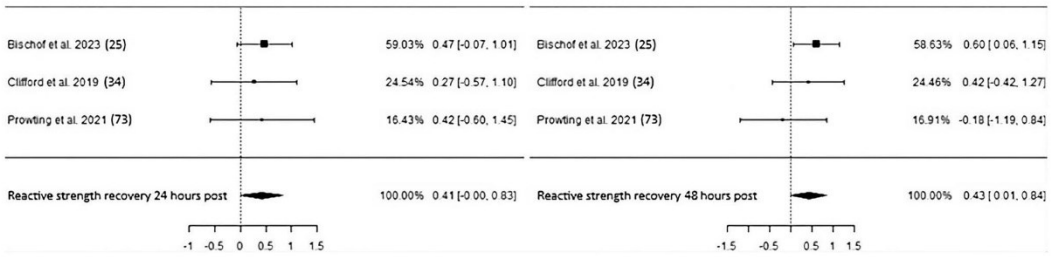


Fig. 8 Studies investigating reactive strength recovery 24 and 48 h following exercise-induced muscle damage with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals

Regarding recovery of reactive strength measured by countermovement jumps 24 h after intense exercise bouts, three studies totaling 91 participants either led to or did not show statistical significance depending on the chosen correlation coefficient ("corr") used to calculate ΔSD when data provision was insufficient (Fig. 8, Table 3). Accordingly, a corr of 0.7 resulted in an ES of 0.45 and p-value of 0.03, whereas a corr of 0.5 resulted in a nonsignificant p-value of 0.05 with an ES of 0.41. Low heterogeneity and no risk of bias were identified in both cases. CP administration also improved reactive strength recovery 48 h following exercise (ES 0.43, $p = 0.045$, CI 0.01–0.84) with low heterogeneity and risk of bias.

Muscle soreness typically evaluated by means of a visual analog scale has not been significantly influenced by CP supplementation in four studies comprising 121 subjects, neither immediately post nor 24 h (4 studies, 127 subjects) and 48 h (5 studies, 135 subjects) after muscle-damaging exercise (post: ES − 0.21, $p = 0.26$, CI − 0.57 to 0.15; 24 h: ES − 0.05, $p = 0.79$, CI − 0.4 to 0.3; 48 h: ES 0.09, $p = 0.7$, CI − 0.38 to 0.57; Fig. 9). Heterogeneity continuously stayed low, and no serious potential of risk of bias emerged (Table 3).

Certainty of evidence Table 4 illustrates the certainty of evidence for each outcome with additional information regarding reasons for downgrading. Fat-free mass can be considered moderate; certainty of evidence of muscle architecture, maximal strength as well as maximal & reactive strength and muscle soreness following exercise-induced muscle damage recovery appears to be low. Very low certainty of evidence exists for tendon mechanical properties and tendon morphology.

4 Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis investigating the effects of longer-term collagen peptide (CP) supplementation in combination with regular physical training on strength, musculotendinous remodeling, functional recovery, and body composition in healthy adults.

Our findings indicate that prolonged CP intake may substantially enhance fat-free mass (FFM) and improve tendon morphology, muscle architecture, maximal strength, and recovery in reactive strength following multiple bouts of muscle-lengthening exercises. However, it is worth noting that the certainty of evidence for any parameter was not evaluated as high.

In line with the findings of two systematic reviews [39, 52], significant favorable body composition changes have been observed following several weeks of consuming CP together with either resistance (RT) or concurrent (CT) training with a moderate certainty of evidence. Furthermore, the current meta-analysis demonstrated enhanced fat-free mass (FFM) along with muscle architectural adaptations, expressed as augmentations in one-, two-, and three-dimensional measurements. Both body-weight and conventional barbell-assisted resistance training (RT) have been shown to stimulate muscle protein synthesis, an effect that is further amplified when additional protein is ingested promptly [53]. Typically, an amino acid composition high in essential amino acids is thought to be crucial for maximizing muscle gains. Leucine, a branched-chain amino acid, is particularly notable for its myocellular signaling ability, enhancing the phosphorylation status of p70S6k, mTOR, and 4E-BP1 [54]. This has led to increases in satellite cell numbers and protein expression of MyoD and myogenin—proteins involved in myoblast differentiation, myogenesis, and the regulation of anabolic balance—when combined with RT in mice [55]. Despite being low in leucine and high in glycine, proline, and hydroxyproline, CPs have also demonstrated the ability to contribute to an increase in FFM. This effect is supported by a moderate level of evidence (one level deducted due to wide ranges of effects) in the current meta-analysis, partly contrasting with previous results.

The present analysis has unveiled a significant increase in muscle mass accrual, measured by thickness, diameter,

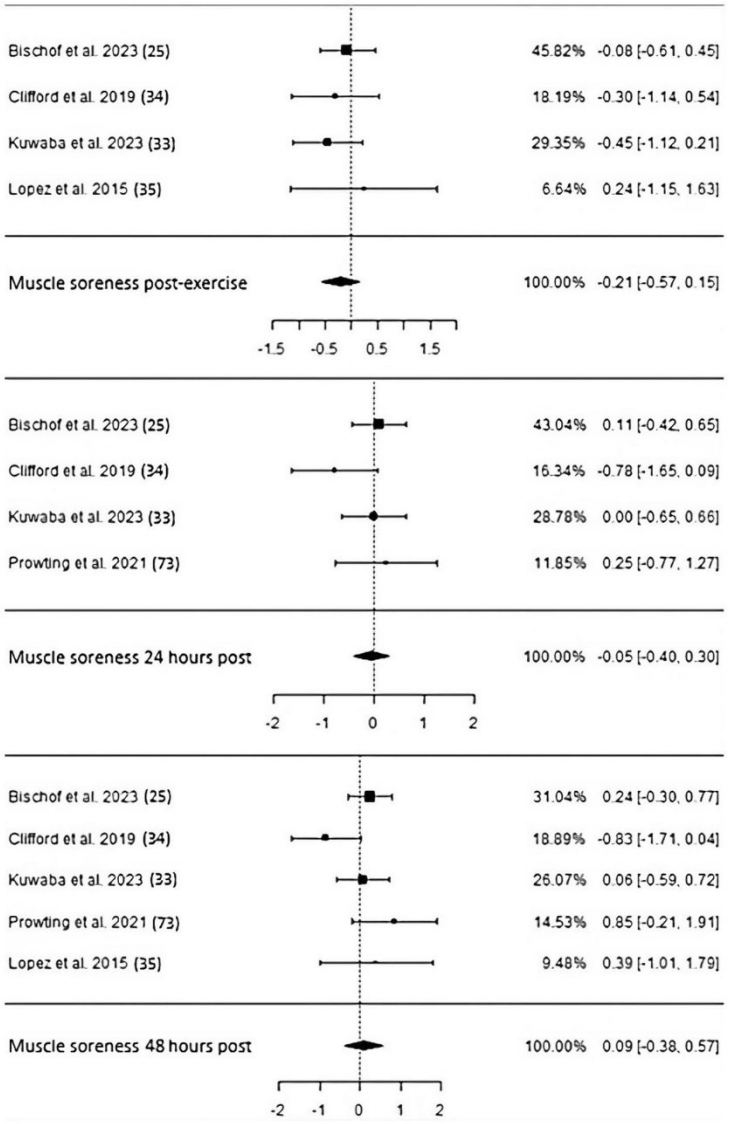


Fig. 9 Studies investigating muscle soreness post, 24 h, and 48 h following exercise-induced muscle damage with individual weights, SMDs (as filled squares), overall SMD (as filled rhombus), and confidence intervals

and volume, albeit with a low certainty of evidence. This low certainty was primarily attributed to indirectness, stemming from differing populations in terms of training status, and imprecision, evidenced by wide confidence intervals. Analogous to leucine, a single portion of CP has been shown to evoke a higher upregulation of key anabolic pathways (PI3K-Akt, MAPK) involved in myofibrillar protein synthesis 4 h following RT [25]. Moreover, an in vitro experiment treating C2C12 cells with the dipeptide hydroxyprolyl-glycine elicited phosphorylation of p70S6k, mTOR, and Akt, indicating myogenic differentiation [56]. So far, only one RCT compared the hypertrophic effects of whey protein and leucine-matched CPs. After 10 weeks of resistance training and daily doses of 35 g/day, whey protein, compared with CP, significantly increased muscle mass in the vastus lateralis and biceps brachii of previously untrained individuals. This suggests that protein sources rich in essential amino acids, like whey, are more

Table 4 Certainty of evidence for each outcome

Outcome	No. of studies	No. of participants	Estimated effect (as SMD)	Certainty of evidence (GRADE)
Fat-free mass	8	418	0.48	⨁⨁⨁◯ moderate[d]
Tendon morphology	4	114	0.67	⨁◯◯◯ very low[b,c,d]
Tendon mechanical properties	4	111	0.05	⨁◯◯◯ very low[a,c,d,e]
Muscle architecture	5	161	0.39	⨁⨁◯◯ low[c,d]
Maximal strength	11	511	0.19	⨁⨁◯◯ low[c,d]
Recovery maximal strength post	3	98	0.15	⨁⨁◯◯ low[c,d]
Recovery maximal strength 24 h	4	113	0.26	⨁⨁◯◯ low[c,d]
Recovery maximal strength 48 h	4	113	0.31	⨁⨁◯◯ low[c,d]
Recovery reactive strength 24 h	3	91	0.41	⨁⨁◯◯ low[c,d]
Recovery reactive strength 48 h	3	91	0.43	⨁⨁◯◯ low[c,d]
Recovery muscle soreness post	4	121	−0.21	⨁⨁◯◯ low[c,d]
Recovery muscle soreness 24 h	4	127	−0.05	⨁⨁◯◯ low[c,d]
Recovery muscle soreness 48 h	5	135	0.09	⨁⨁◯◯ low[c,d]

Reasons for downgrades: a = risk of bias, b = inconsistency/heterogeneity, c = participants and/or interventions differed (training history, duration, study design), d = imprecision/ wide confidence intervals, e = publication bias (detected via Egger's test)

SMD standardized mean difference

effective than CPs for increasing muscle thickness [57]. Additionally, it is worth mentioning that the studies primarily focused on muscle groups in the lower limbs, with a noticeable lack of data on muscle adaptation in the upper limbs. Future experiments should consider the involvement of upper-body muscles, as varying inter-muscle mass adaptability has been observed in several long-term exercise trials [58–61]. Therefore, it remains to be determined whether CP intake also influences muscle growth in the upper body.

In line with the current findings demonstrating a small yet significant overall benefit from CP supplementation on maximal strength, it is commonly observed that muscle mass accrual is associated with enhanced strength [62]. This association can be attributed to the fact that myofibrils are the primary force-producing units within the musculoskeletal system. Additionally, factors such as muscle fiber composition, muscle architecture, neural activation of agonist and antagonist muscles, and specific tension also contribute to increases in muscle strength. Balshaw and colleagues presented significantly higher evoked contractile twitch peak torque after chronic CP intake but no group-specific quadriceps tension and muscle architectural differences. Only the pennation angle of the vastus intermedius (among all other quadriceps muscles) showed a tendency to have increased over a 15-week RT period [28]. Furthermore, the results of another study failed to demonstrate a relative and absolute muscle fiber type and group specific adaptation following 15 g CP together with 12 weeks of RT, possibly justified by the already RT experienced cohort and their already high protein intake of > 1.7 g/kg/day. The authors hypothesized that the observed changes were more likely due to adaptations in passive tissue components rather than myofibrillar accrual [51]. Nevertheless, another 12-week RCT investigating older sarcopenic men undergoing RT plus daily 15 g of CP led to significantly higher strength output in the CP group, outlining CPs' potential effects in non-RT-trained individuals [24].

As shown in the current meta-analysis, tendon CSA (mostly comprising patella tendons) was significantly increased following long-term RT with CP intake, indicating a pronounced tendon remodeling particularly after several months of exercising. This is in accordance with a comprehensive systematic review and meta-analysis highlighting most beneficial morphological tendon changes after 12-week training interventions, regardless of muscle contraction types (concentric, isometric, eccentric) [63]. The potential superiority of CP supplementation might lie in the enhanced collagen synthesis of force-transmitting passive tissues. Blood sera from subjects who performed 6-min rope-skipping sessions combined with 15 g of gelatin have been shown to significantly increase collagen concentration in human-engineered ligaments compared with 5 g and a placebo. This increase was accompanied by elevated levels of the N-terminal peptide of pro-collagen I (P1NP), a marker of collagen formation. In parallel, collagen-specific amino acids glycine, proline, hydroxyproline, and hydroxylysine were significantly elevated up to 2 h after gelatin ingestion in the subjects' blood [7]. Absorption experiments in human blood have also affirmed rising levels of collagen-specific amino acids, di- and tripeptides [8, 64, 65], achieving even higher uptakes when ingesting low-molecular-weight peptides (~ 3 kDa and below) derived from collagen hydrolysates [66]. PEPT-1, an

active transmembrane peptide transporter located at the intestinal luminal brush border, may be responsible for the uptake of di- and tripeptides, and some of them, containing hydroxyproline and/or proline, might withstand cytosolic peptidases so that intact entry into the blood stream is feasible [5]. Lastly, since high loads from physically demanding activities apply enormous tendon stress, a large tendon CSA may act in an injury-preventive manner from a physical point of view (stress = force/CSA) [32]. Based on the current findings, CP supplementation might help adolescent athletes balance out muscle and tendon disparities by promoting tendon CSA growth to match muscle growth that is typically seen in adults [67]. However, the level of evidence appears to be very low, elicited by concerns of heterogeneity, study population differences, and wide effect ranges.

Somewhat surprisingly, tendon mechanical properties revealed no difference between CP and placebo in the present analysis although significant morphological adaptations occurred. Tendon stiffness (ratio of tendon elongation to tendon force applied) and Young's modulus (stiffness multiplied by the ratio of tendon length over CSA) as parameters defining tendon mechanical properties in the present analysis have already been reported to be significantly increased with no concomitant changes in tendon CSA [68]. A recent systematic review and meta-regression also revealed that an increased modulus rather than the CSA was the predominant moderator of enhanced tendon stiffness. The main reason for the rise in tendon stiffness due to training is thought to be an increase in gene expression related to anabolic responses to strain, leading to, seemingly lower, collagen synthesis and turnover. Additionally, enhanced enzymatic cross-linking of collagen might also play a role [69] as well as noncollagenous extracellular matrix (ECM) components (e.g., glycoproteins, elastin) which may reduce energy loss and help recover stored energy [70]. Stiffness and Young's modulus are supposed to be highly dependent on load intensity and contraction type, with eccentric ones being superior [68]. In one out of four included studies in the current meta-analysis, subjects performed body-weight and plyometric exercises instead of high-load RT [41]. In addition, two of four studies recruited already (resistance) training-experienced individuals [30, 41], emphasizing the challenges in identifying significant effects attributed to a well-developed training level.

Muscle soreness as a frequently used indicator of muscle damage was usually gauged several times within 48 h following muscle damage-inducing exercise (MDIE) in the current meta-analysis without any significant effects. Participants of included studies documented their perceived muscle soreness immediately following either countermovement jumps (CMJ) or squats (of note, two [38, 71] out of five studies did not provide this information). Activity-related pain could have influenced the results of CMJ or squat exercises, both of which involve a wide range of motion. CP has already been effectively utilized to notably decrease pain during physical activity after a 12-week regimen of 5 g of CP daily in athletes experiencing exercise-related knee pain [72, 73]. However, there was no significant difference in muscle soreness reported, indicating that only subjective pain was recorded, which may not be influenced by long-term CP intake. As muscle soreness is also closely related to inflammation [74] and expresses the degree of muscle damage and/or regeneration at least to some extent [75], animal studies have demonstrated anti-inflammatory potential of CPs and glycine alone. CPs were able to positively modulate local and systemic inflammatory response by reducing swelling and interleukin-6 (IL-6)-mediated lipopolysaccharide (LPS) production, possibly via the activation of glycine-gated chloride channels [76]. A solely glycine administration also has been reported to decrease plasma and mRNA tumor necrosis factor-α (TNF-α) levels as well as reduced muscle mRNA expression of Toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain protein 2 (NOD2), two major proteins involved in inflammatory signal transduction [77]. In a short-term human RCT, a 1-week daily CP supplementation was not able to alter IL-6 levels compared with a placebo following several bouts of muscle-damaging exercises [37]. Therefore, it remains to be investigated if and how CPs could influence human muscle soreness.

In the present analysis, elevated regeneration of maximal strength for each time point (post, 24 h, 48 h) lacked statistical significance indicating no influence of CP intake in this respect. Performed tests (biomechanical-related exercises such as CMJ or knee extensions performed on a force-capturing dynamometer) typically lasted seconds and subjects required 0.5–2 min of rest. Considering methodological aspects, a study conducted on minimal rest period between maximal voluntary contractions concluded that a 60-s rest interval is sufficient but seems mandatory for the regeneration of force-producing capacity in healthy individuals [78]. A shorter rest period thus might have influenced the outcome. Reactive strength (measured by means of CMJ height) displayed a tendency to reach significant effects at 24 h (depending on the correlation coefficient used to calculate ΔSD) and eventually did at 48 h (ES 0.43, $p = 0.045$), which underlines a certain correlation between these two types (maximal and reactive) of muscle strength [79]. The results might be difficult to interpret since one investigation sought to determine differences before and following an implemented 12-week CT [35] whereas others did not include any training intervention and therefore administered CPs to a lesser time extent (1–6 weeks). One possible explanation of CPs showing a positive trend/effect here appears to be the chronic adaptation of tissues that possess the capability to store and release elastic energy during muscle contraction such as actin and myosin filaments and their formed cross-bridges, titin, and the

connective tissue scaffolding of the ECM. Regarding tendons, it is known that the retention and release of elastic energy can significantly influence the force, power, and velocity of movement [80]. In general, the ECM is naturally stiffer than muscle fibers and plays a significant role in generating passive force within muscles. Hence, the observed effects of greater improvements in reactive strength compared with maximal strength in the current meta-analysis could be attributed to the positive influence of the stretch–shortening cycle during countermovement jumps (CMJ), a factor less influential during maximum voluntary contraction tests [71]. Another speculative reason for enhancing performance regenerative capacity could be attributed to the reduction in oxidative stress and inflammation associated with CPs. Glycine (the most abundant amino acid in CPs) acts through glycine receptors located on several immune-related cell types. It has been reported to attenuate superoxide production in neutrophil granulocytes [81] and may decrease the synthesis of pro-inflammatory mediators while inhibiting LPS-induced cytokine production [82]. Recently, CP's potential ability to reduce endotoxemia [measured via lipopolysaccharides (LPS)] after gastrointestinal stress induced by intense aerobic exercise has been demonstrated in humans [83]. A recent review also revealed hydrolyzed CP to have possible anti-inflammatory and chondroprotective effects in patients with osteoarthritis, but considerable limitations exist due to methodological disparities [84]. Furthermore, most of the supplementation interventions lasted > 3 months, whereas the periods in the current analysis regarding strength regeneration were of much shorter durations. It remains to be elucidated which underlying factors and mechanisms led to such CP associated short-term adaptations since force transmitting tissues (e.g., intramuscular connective tissue and tendons) are unable to adapt within a week. Also, significantly increased collagen formation accompanied by several days of CP intake has not been reported (supported by blood levels of P1NP [37]).

Strengths and limitations One of the major strengths in this review demonstrates the overall low risk of bias with no studies having ranked less than "good" (PEDro score). However, future investigations are encouraged to conduct additional intention-to-treat analyses and report end-of-study ("post-intervention") data of as many participants starting the trial as possible to further diminish risk of bias. In addition, most of the trials (12 out of 19) administered 15 g CP that is currently expected to elicit the most beneficial sports performance and injury-preventive results. Nonetheless, a recently published study suggests to provide 30 g instead of 15 g CP to further enhance collagen synthesis response, at least in resistance-trained subjects following an acute RT bout [85], also highlighting the urge for CP dose–response experiments. Based on previous investigations applying varying protein dosages in active individuals, at least muscle protein synthesis has been significantly increased using more than 15 g (20 g after RT [86] and ~ 0.5 g/kg body weight following endurance training [87]), surmising that also greater doses (> 15 g) of CP are required to act maximally stimulating in experienced athletes.

Although statistical effects throughout this meta-analysis have been identified, the certainty of evidence using the GRADE approach was moderate to very low for significant outcomes. This uncertainty stemmed from several factors, including the indirectness of evidence, as subjects sometimes differed in their training status, resulting in heterogeneity in musculoskeletal adaptability. Imprecision of results through "unfavorable" distribution of confidence intervals, risk of bias, heterogeneity (as indicated by a higher I^2), differing types of intervention, and their length (in recovery-associated studies) and potential publication bias as indicated by a significant Egger's test can also be constituted as downgrades. Apart from that, not all authors were able to share change scores of the standard deviation of their trials and respective subjects that consequently caused using a specific correlation coefficient ("corr") to calculate the ΔSD of three studies. Depending on the corr, the results changed markedly, as demonstrated by the significant recovery in reactive strength observed 24 h post-exercise (corr 0.5 → $p = 0.051$; corr 0.7 → $p = 0.033$). Overall, the number of studies included for tendon- and recovery-related parameters was low, indicating further future research in this field.

The magnitude of musculoskeletal adaptability is highly dependent on individual training history as well as age. Two included studies were completed by older subjects (60+), with one even recruiting sarcopenic elderly subjects, which might have influenced at least some outcomes. Moreover, the integration of highly trained athletes involved in two trials might have left some impact on the results since musculotendinous remodeling happens at lower rates compared with untrained/recreationally active subjects, therefore possibly reducing effects of the intervention. Lastly, three experiments added additional vitamin C to CP supplementation, expecting to increase hypothetical adaptational processes in collagen-containing tissues, given that vitamin C is an essential cofactor of lysyl hydroxylase and prolyl hydroxylase, both indispensable for collagen biosynthesis [88]. However, there has not been any CP + training intervention trial conducted so far that compared both CP + vitamin C and CP alone.

5 Conclusion

Prolonged collagen peptide (CP) supplementation combined with resistance or concurrent training appears to be a beneficial adjunct for healthy active adults and athletes striving for an increase in fat-free mass and maximal strength and an improvement in tendon morphological properties and reactive strength recovery. CP's potential ability to further

increase tendinous cross-sectional area may represent an effective option to anticipate sports-related tendon injuries. The current rationale to induce aforementioned adaptations seems to be a daily dose of 15 g CP for at least 8 weeks. Acute supplementation patterns (1–6 weeks) could also aid in enhancing regenerative capacity, but underlying mechanisms behind possible effects remain unclear. Future investigations are encouraged to figure out the optimal CP dosage and composition as well as their effective absorption and incorporation into force-producing and transmitting tissues. Moreover, experienced athletes might profit from higher CP (> 15 g) dosages as recently reported in other trials investigating protein supplementation and its effect on enhanced protein synthesis. In particular, individuals participating in sports known to exert significant stress on tendons (such as powerlifting and team sports involving uncontrolled movements like soccer and tennis) could potentially derive the greatest benefit from several months of collagen peptide use.

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Declarations

Authors' contributions D.K. and K.B. designed the review; A.M.M. and K.B. performed screening and study quality assessment; K.B. and SS analyzed the data; K.B. wrote the manuscript; A.M.M., D.K., K.B., and S.S. reviewed the manuscript. All authors read and approved the final version.

Availability of data and material The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation upon request.

Code availability No self-written code was used for the meta-analysis. The freeware Jamovi for Microsoft Windows 11 (https://www.jamovi.org/download.html) performed all meta-analyses and created all forest plots.

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Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3. Methodological considerations

While the methods used are, of course, stated in each paper (section 2), this chapter will provide a more detailed discussion of their suitability in the context of the papers, along with their limitations and possible alternatives.

3.1 Methods used in paper 1

3.1.1 Randomization

Research randomizer, a simple and easy to use online tool (https://www.randomizer.org/), was used to randomly allocate participants to either the placebo or the treatment group. Furthermore, concealed allocation was ensured to blind the investigator during assignment of subjects to a specific study arm. To maintain a double-blind trial design, a representative from the supplement manufacturer unblinded the study at its conclusion.

3.1.2 Study design

In order to gauge regeneration of human physical performance-related (bio-) markers, consecutive measurements throughout a predetermined period of interest are usually carried out. Considering the duration of muscle damage and soreness in non- to little-exercised subjects, choosing a time window of up to 12 days could be appropriate to see differences in groups. This assumption is supported by a human trial that, even after 11 days following eccentric exercise bouts of the elbow flexors, still did not observe a return to baseline levels in muscular pain, swelling, and strength (132). However, recovery-related studies conducted so far (as mentioned in section 1.8) majorly experienced peaks within 48 hours after muscle-damage induced exercised, which is why (+ economic reasons) a time window of 48 hours was also determined (overview of the study design is illustrated in Figure 3).

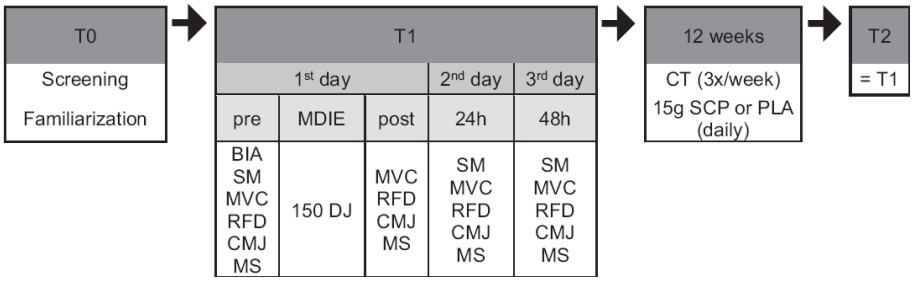


Figure 3: Study design in chronological order. BIA, bioelectrical impedance analysis; MVC, maximum voluntary contraction; RFD, rate of force development; CMJ, countermovement jump; MS, muscle soreness; MDIE, muscle damage-inducing exercise; DJ, drop jump; CT, concurrent training; SM, standardized meal; (119).

3.1.3 Muscle damage inducing exercise

150 drop jumps have been performed by the participants to induce substantial muscle damage. These were split into six sets of 25 repetitions with a two-minute interest break and carried out from a 60 cm box as seen elsewhere (114). Emphasis was placed on slow and

controlled movements, ensuring a knee angle of at least 90° upon landing to highlight eccentric contractions in this exercise, which likely induces muscle damage and soreness, especially since the subjects were unaccustomed to it. In principle, multiple sets of eccentric exercises likely cause muscle damage in untrained subjects who are not accustomed to them. Barbell-assisted exercises like squats or bench presses could also be used; however, since these exercises require some level of training experience to prevent injuries, drop jumps were chosen as a suitable and effective alternative for the study, being relatively easy for this cohort to perform.

3.1.4 Dietary intake

Dietary intake throughout the trial was monitored by means of food tracking of two weekdays and one weekend day, at the beginning and the end of the study. From the weight/volume data of food/drinks consumed by participants, total energy, fat, protein and carbohydrate intake were calculated. Some studies also measured vitamin C intake (when adding vitamin C to collagen peptide supplementation), likely to ensure that its levels were sufficient and did not affect collagen synthesis. As already discussed in section 1.6.1, vitamin C deficiencies were not expected in participants living in Austria (due to vitamin C being the most fortified nutrient in processed food (133) and nutritional supplements are not necessary for sufficient vitamin C status (134)), thus this micronutrient was not included in the nutritional analysis.

3.1.5 Standardized meal, supplementation and forbidden substances

A standardized meal was provided during the three consecutive test days (at the beginning and end of the study), which consisted of oats and an oat drink mixed at a ratio of 1 gram per kilogram of body weight. Depending on the subject number, meals where ingested between 6:40 and 9:00 a.m. following an overnight fast and at the same time 12 weeks later. It was ensured the participants received their meals two hours before the test session began. In addition to alcohol prohibition 48 hours prior to the start, some (135, 126) but not the majority of studies also forbade caffeine intake the day before. Caffeine appears to positively influence neuromuscular performance, but these effects are more likely to be seen when caffeine is administered immediately before exercise (136). The use of other ergogenic supplements such as creatine was also prohibited (perpetually).

The test product given to the treatment group consisted of a 15 g mixture of collagen peptides and was provided by a German manufacturer, as was the metabolically inert, non-energetic placebo. The participants received sachets of 15 g of their assigned products, which were similar in taste and texture. Participants were required to ingest a sachet with water at the same time every day, and on training days, they consumed half a portion one

hour before and immediately after the training to ensure high levels of absorbed peptides in the blood around the time of exercise, ensuring an effective utilization of the subsequent “anabolic window” (137, 138). Other approaches exhibited a morning and evening intake (without accompanied exercise intervention) (113), ingestion of one daily portion one hour before (93), immediately (127, 128) and one hour after training (9), emphasizing a peri-exercise collagen peptide ingestion regimen significance.

3.1.6 Body composition

A bioelectrical impedance analysis (BIA) (Nutriguard MS, data-input GmbH, Germany) obtained changes in body composition while subjects previously laid horizontally on a mat to guarantee optimal body fluid distribution as described by the manufacturer. Parameters of interest were fat mass, fat-free mass (muscles, organs, skeletal, nervous system; comprises extracellular and body cell mass), extracellular mass (collagen-rich tissue; mainly skin, tendons, fasciae, and bones) and body cell mass (metabolically active tissue; mainly muscles and inner organs) (139). Other methods of body composition measurement include air displacement plethysmography, magnetic resonance imaging, computed tomography and dual-energy X-ray absorptiometry, all being capable of gauging total fat and lean tissue while the last both use ionizing radiation, limiting their application in healthy subjects (140). No method can directly quantify fat- and fat-free mass in vivo. Thus, all methods are inherently reliant on assumptions, whose accuracy significantly varies depending on patient characteristics like sex, age and their nutritional status (141). However, following specific guidelines prior to testing may enhance accuracy of body composition measurement. It has been recommended to do an overnight fast, to stop drinking two hours before commencement, to abstain from exercise within 24 hours and from alcohol within 48 hours (142). Given the consistent adherence to these recommendations, along with considerations of financial and logistical factors, BIA was deemed the most suitable method for the study.

3.1.7 Exercise intervention

A 12-week concurrent training intervention, combining both endurance and resistance exercises, was conducted outdoors on a running track also due to COVID-19 regulations that made indoor training difficult. The first part of the ~ one hour lasting training session, which took place three times a week (Monday, Wednesday, Friday), comprised three sets of 20 repetitions of squats, lunges and calf raises, increasing to 25 and 30 repetitions after four and eight weeks, respectively, and with interest breaks of two minutes. Afterwards, a 30 minute run at an individually predetermined heart rate (HR) was performed with continuous tracking of pace and heart rate via a smartwatch. The formula of Karvonen (143) calculated individual HRs for each month, beginning with a training factor 0.7 to 0.8, and increasing

0.05 each month, providing a moderate to high training intensity (training HR = resting HR + (maximal HR – resting HR) * training factor [0-1]; 220 - age calculated max. HR). Alternative accurate methods to prescribe training intensity are based on ventilatory or lactate thresholds obtained by spirometry, which has already been applied in studies investigating collagen peptide effects on cardiovascular performance (126, 135). As endurance outcomes were not of particular interest in paper 1, the Karvonen formula seemed quite appropriate for prescribing training with respect to the included cohort. In general, the selection of concurrent training as the training intervention leverages multiple physiological adaptations and benefits, representing a synthesis rather than a mere compromise of the outcomes typically achieved through resistance or endurance training alone. A recent systematic review with meta-analysis reported concurrent endurance and resistance training does not hinder the development of maximal strength and muscle hypertrophy compared to strength training alone, regardless of the endurance training type, the frequency, training status or age group. However, explosive strength might suffer from aerobic training (144), which is why resistance training was performed prior to endurance training, a constellation already shown to increase one-repetition maximum compared to its reversed order (145). Moreover, concurrent training was reported to significantly enhance cardiorespiratory fitness (often expressed as VO2max) compared to RT alone (146, 147). The prescribed concurrent training intervention also highly contributes to reaching the current recommendations of the World Health Organization (148). Based on these considerations and findings, concurrent training seemed very suitable for exercise-induced adaptations.

3.1.8 Primary parameters

Primary parameters of paper 1 included maximum voluntary contraction (MVC), rate of force development (RFD), countermovement jump (CMJ) height and muscle soreness. The first two were performed on an isokinetic dynamometer (HUMAC NORM Model 770; CSMi, Stoughton, MA, USA) with participants secured by seatbelts and straps for the upper and lower body, while keeping their arms crossed over the chest to ensure standardized testing positions. The knee angle (110° for maximum torque) was individually adjusted at baseline and maintained for all subsequent measurements. The hip angle remained approximately 110° for all subjects. Following the countdown “3, 2, 1, go,” participants extended their right leg as forcefully as possible for 3 seconds during the MVC trials. For the RFD trials, subjects were instructed to contract their right leg explosively (as fast and as forceful as possible), generating maximum force within a very short duration (< 200 ms) (149). Strong verbal encouragement was provided to the subjects during the contractions while offering simultaneous visual feedback. The analog signal of joint moment was recorded using the Vicon Nexus A/D card (16-bit) at a sampling rate of 1,000 Hz. The data were then filtered with a

cut-off frequency of 20 Hz (150). A valid MVC trial required the force to be maintained consistently for at least 2 seconds. The average of the maximum force (N) from three valid trials was then used for further analysis. When measuring the rate of force development, any countermovement of the tested leg was forbidden and led to exclusion from the trial. RFD was calculated as the slope of the force-time curve (Δforce/Δtime) over the 0–50 ms time interval. Force onset was automatically determined when the force signal reached a threshold of about 1.4% of MVC, following a method similar to that described in the literature (151). The automated onset detection produced higher intraclass correlation coefficients (ICC) and lower percentages of the standard errors of the mean (SEM%) in comparison to manual onset detection (152). Apart from RFD, peak RFD (0-100 ms) was also obtained during the trials and is considered a further reliable parameter (153). Following MVC and RFD trials, CMJs were carried out barefoot with both feet about shoulder-width apart on a single force plate. A CMJ was initiated with a countermovement, typically characterized by a knee angle of approximately 90 degrees, though individual movement patterns and angles were also accommodated to some extent. Participants kept their hands on their hips throughout the entire movement. After the countermovement, the goal was to jump as explosively and as high as possible. The impulse-momentum method $J = J_{GRF} - J_{BW} = m * v_{take\text{-}off}$ leading to $y_{flight} = y_{peak} - y_{take\text{-}off} = (v_{take\text{-}off}^2) / 2 * g$ calculated CMJ height (J_{GRF} is the impulse due to the ground reaction force and J_{BW} due to the jumper's body weight) (154). As for all other biomechanical parameters, the mean of three valid trials was used for further analysis, as this approach appears to be more sensitive in detecting fatigue than using the maximum value (155), and a one minute between trial break was maintained in order to guarantee fully recovered subjects for each attempt (156). The individual subjective perception of muscle soreness was assessed after the third valid CMJ attempt using a simple visual analog scale, consisting of a ruler with a slider ranging from 0 to 10 cm, marked with 1 mm increments.

Regarding the physical exercise tests, a crucial part of the study was the familiarization session, which occurred approximately two to three weeks before the study began. This session allowed participants to become acquainted with the lab equipment and practice for the upcoming tests. When deciding to choose these particular tests/ parameters for the study, the idea was to implement surrogate markers of maximal, explosive and reactive strength combined with a rather subjective marker providing information about individual delayed onset muscle soreness to cover multiple aspects of fatigue. Moreover, it was assumed that if collagen peptide supplementation impacts exercise-related recovery, this would likely be evident through specific exercise tests. The outcomes of these tests would depend on the condition of collagen-rich tissues (e.g., muscles, tendons, myotendinous

junctions) and their role in efficient force transmission, as long-term uptake of certain collagen peptides was expected to happen, thereby presumably strengthening corresponding tissues.

Regarding MVC, the length of the RFD recording period is essential to obviate redundant data as late RFD is strongly correlated with MVC when performed longer than 200-250 milliseconds. Late RFD/ MVC are influenced by musculotendinous stiffness, fiber type distribution and properties of muscle architecture whereas early RFD (typically < 100 ms) is mainly determined by motor unit discharge and recruitment rate – that is neuromuscular activation (157, 158). Noteworthy, RFD, compared to MVC, is more closely related to the performance of both sport-specific activities and functional daily tasks but lacks high reliability especially during the early contraction phase (149). Based on aforementioned assertions, MVC and (early) RFD appear to be suitable parameters, covering multifaceted aspects of strength. In addition, practical recommendations from a review, which thoroughly addresses RFD methodological considerations (149), were continuously followed to ensure a reliable and valid data output.

The countermovement jump (CMJ) as the chronologically last exercise in the test battery portrays an easy to perform and subject-friendly test that is highly associated with reactive strength of the lower limbs, and CMJ height is correlated with speed, agility, power and explosive strength (159). Since the CMJ movement pattern heavily relies on the stretch-shortening cycle to achieve maximum heights, tendons that can store high levels of elastic energy are essential (160). Accordingly, if collagen peptides incorporate in and strengthen tendinous tissue (enhancing also stiffness), CMJ height appears to be a practicable indirect exercise test/ marker.

Concerning muscle soreness, other methods such as the pressure pain threshold, have also been used in the literature to assess muscle soreness (114, 161). The pressure pain threshold, defined as the point where a non-painful pressure stimulus turns into a painful one (162), measures localized muscle pain by using a handheld algometer to apply pressure to a specific muscle site. Furthermore, the perceived exertion rating Borg scale, which is available as a 9-, 15-, 21-point and category-ratio scale (163), could also be used as it demonstrates high and similar reproducibility as the visual analog scales; both also being superior to the 5-point likert scale (164).

3.1.9 Statistical analyses

IBM SPSS Statistics 23 (IBM SPSS Statistics for Windows, Armonk, NY, USA: IBM Corp.) carried out all statistical analyses. An α level of 0.05 was established, normal distribution was assessed using the Kolmogorov-Smirnov test, and homogeneity of variance was

checked with Levene's test. As three factors were present in the study, namely group (placebo, collagen), intervention time (before and after 12 weeks) and recovery time (pre, immediately post, 2h, 24h, 48h post exercise (= drop jumps)), a mixed ANOVA (analysis of variance) was performed. For baseline anthropometric data, an independent t-test was used for analyzing between group effects (placebo vs. collagen). Following ANOVA, the partial eta squared (ηp^2) was constituted as effect size (small effect: $\eta p^2 > 0.01$, medium effect: $\eta p^2 > 0.06$, large effect: $\eta p^2 > 0.14$) (165). Mauchly's test of sphericity was also checked. If significant, an ε of < 0.75 led to the Greenhouse- Geisser correction, an $\varepsilon > 0.75$ to that of Huynh Feldt (166). Due to groups being fairly equal in number in the mixed ANOVA (largest/smallest < 1.5), demonstrating F-statistics robustness (167), the homogeneity of variance was not checked in the ANOVA. To reduce the risk of bias, an intention-to-treat (ITT) analysis (= dropouts included in the statistical analysis) could have been conducted. However, limitations have been reported regarding ITT (168) and multiple ITT methods exist that may be used interchangeably without a clear rule of thumb (169).

3.2 Methods used in paper 2

As paper 2 (section 2) included the same study design (test procedures) as paper 1 but with blood sampling, only additional information with respect to statistics and blood collection will be provided in this subchapter.

3.2.1 Statistical analyses

In addition to the statistical analyses used in paper 1, an effect size in independent t-tests was given as Cohen's d (small effect: >0.2, medium effect: >0.5, large effect: >0.8) (165). Since high variability in blood parameters may and have been observed, data above the third standard deviation were not taken into account. Determining outliers remains a complex endeavor, as several (>30) methods exist and each of them might influence the outcome of a data analysis (170). The area under the curve (AUC) by means of the linear trapezoidal rule for each blood parameter (171) as well as the deltas of these AUCs were calculated to elucidate total individual levels within 48 hours following exercise-induced muscle damage.

3.2.2 Blood collection & parameters

Each blood sample was drawn via venipuncture and collected in 10 mL serum vacutainers. The samples were then centrifuged at 2500 rpm for 15 minutes, after which the resulting supernatant was transferred into 2 mL Eppendorf tubes and immediately stored at -32°C. For analysis, the frozen tubes were transported to a local laboratory where they were ana-

lyzed individually. The Beckman Coulter AU5822 analyzer (172) was used to measure creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (MYO). High-sensitivity C-reactive protein (hsCRP) was measured at "pre" and "24 h" time points using immunoturbidimetry (Alinity c CRP Vario Reagent Kit, Abbott Laboratories) with a coefficient of variance (CV) of 12.5%. The 24-hour measurement was selected due to the high likelihood of CRP levels peaking at 24 hours post-exercise (173, 174).

CK, LDH, MYO and CRP have been extensively used as metabolic and/ or recovery-associated biomarkers for monitoring muscular fatigue in athletes (175, 176). CK typically increases after unfamiliar eccentric exercise bouts that place mechanical stress on muscle cells, leading to membrane damage. This allows CK to leak out of the cells and into the bloodstream, where its activity can be quantified (177). The augmented myocellular permeability and microtrauma resultant CK commonly peaks within 48 hours post-exercise, depending on the type of training (175).

LDH resides in almost every tissue and catalyzes the reversible conversion of pyruvate to lactate with the accompanied conversion of NADH to NAD+. As myofibrillar tissue is highly dependent on fast energy utilization, it can be assumed that most of the LDH in blood derives from exercise-induced stress. LDH usually peaks within 24 hours following intense exercise and thus appears to be a practical readily available marker (178). Furthermore, a high reliability has been reported after a 60-minute endurance exercise (179).

MYO, a muscle protein, which contains a heme group, plays a pivotal role in intramuscular oxygen transport by capturing oxygen from blood hemoglobin and delivering it to oxidation sites within the muscle. When muscle cells are damaged, their membrane permeability presumably increases, enabling MYO to escape cellular membrane. Analogous to LDH, MYO typically reaches its peak concentrations within 24 hours post-exercise, therefore also serving as a rather promptly available recovery marker (177, 175).

CRP, an acute phase protein produced in the liver, also responds to exercise associated muscle damage induced inflammation (176). Inflammatory cytokines such as IL1β and IL6 trigger the production of CRP (178). CRP levels typically exhibit peak levels at 24 hours post-exercise as also reported following an ironman race (180). As CRP is commonly used to inform about systemic inflammation, prolonged physical exercise may convey a reduction in CRP levels (181).

Based on the above given information, the idea of paper 2 was to use blood parameters that are capable of exhibiting different time courses and processes of recovery. However, as previously reported in a comprehensive review, the aforementioned biomarkers have certain limitations (178): Inter-individual variability is considered high, especially in CK values. LDH as mentioned before is not muscle tissue specific. MYO appears to be a rather short-term recovery marker, making it an unfavorable parameter for monitoring long-term

recovery processes. Elevated CRP levels may not only occur following intense exercise, but also as a result of clinical conditions.

3.3 Methods used in paper 3

Paper 3 portrays a systematic review with meta-analysis where pooled evidence of mainly long-term data derived from trials administering collagen peptides in combination with regular exercise was calculated. Given the comprehensive explanations of methods used in the review, three concerns related to the systematic review will be discussed below to avoid redundancies.

The updated version of AMSTAR (A MeaSurement Tool to Assess systematic Reviews), namely AMSTAR-2, demonstrates a currently widely-used instrument to critically appraise systematic reviews including RCTS and non-randomized studies of healthcare interventions based on 16 items without an overall score (182). The rating of overall confidence of a review depends on addressing these 16 items of which seven are stated critical domains. The number of insufficiently or not at all addressed domains (of both critical and non-critical) implicates whether the corresponding review illustrates high, moderate, low or critically low confidence. Based on AMSTAR-2, paper 3 equals a moderate confidence due to failing to address two minor domains. In summary, data extraction was not conducted by two reviewers, and the funding sources of the included studies, if available, were not reported. Nonetheless, AMSTAR-2 is based on expert opinions, seeks for feedback in other research settings and has not been validated extensively (182). Moreover, recent recommendations have suggested modifications to certain items (183).

A variety of tools is currently available to accurately assess risk of bias of included studies. The Physiotherapy Evidence Database (PEDro) scale was applied due to its close proximity to physical therapy/ exercise interventions and therefore suited well for the analyzed studies. A meta-epidemiologic study indicated that researchers could use either the Cochrane Risk of Bias (RoB) tool or the PEDro scale, as neither is regarded as the definitive standard for assessing risk of bias (184). Cochrane's updated version RoB 2 was released in 2019 and appears to be also a viable instrument with recently reported recommendations derived from its pilot phase (185). Another tool, recently uploaded on open science framework as a preprint version, is the SMART-LD (Standards Method for Assessment of Resistance Training in Longitudinal Designs) instrument designed to assess risk of bias and quality of reporting in longitudinally conducted resistance training trials (186). However, since no information is currently available regarding its reliability and validity, it may be premature to use this tool in systematic reviews.

The last point to mention is the application of the adapted/ extended PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement, namely the

PERSiST (Prisma in Exercise, Rehabilitation, Sport medicine and SporTs science) guidance that has been followed throughout paper 3. PERSiST implements PRISMAs 27 items in systematic reviews of sport and exercise medicine, musculoskeletal rehabilitation, and sports science to avoid poor reporting in this research area. The corresponding article offers valuable considerations, resources, suggestions, and examples of reporting (187). Thus, future works are encouraged to apply the PERSiST approach in exercise science related systematic reviews.

4. Discussion and outlook

The results of paper 1 and 2 suggest that collagen peptide supplementation significantly improves the repeated bout effect, a recovery-associated phenomenon linked to reduced muscle damage (and thus muscle damage markers) following a second bout of originally unaccustomed exercise (after days/ weeks of recovering from the first bout) commonly observed within 24 to 48 hours post-exercise (188). This muscle-inherent protective mechanism typically seen after bouts of muscle-lengthening contractions (e.g. eccentric resistance training or downhill running) appears to be evoked by inflammation, ECM remodeling, neural and musculotendinous adaptations (189). Indirect evidence from papers 1-3 suggests that long-term effects of collagen peptide supplementation are not involved in certain inflammatory responses (paper 2). However, they have been shown to influence the remodeling of the ECM (paper 3, evidenced by an increase in fat-free mass, which includes extracellular and body cell mass), neural adaptations (paper 1, indicated by an increased rate of force development), as well as improvements in muscular and tendinous tissue (paper 3, demonstrated by significant enhancements in muscle architecture and tendon morphology). Direct evidence from a 12-week resistance training trial also referred to upregulated ECM proteins such as Fibrocentin, Laminin and several collagen type-specific α chains (109), facilitating cell attachment, being involved in myogenic differentiation, providing tensile strength and controlling cell adhesion, respectively (190, 191). Findings from an in-vitro experiment point to a possible involvement of hydrolyzed collagen in inflammation by attenuating lipopolysaccharide-induced TNFα, IL1β, IL6 and IL8 levels (= pro-inflammatory cytokines) (192). It currently remains elusive how exactly, whether pro- or anti-inflammatory, the human organism responds to a second muscle-damaging bout from an immunological point of view since equivocal findings have been reported (189). Nonetheless, an initial pro-inflammatory phase followed by a subsequent transition to an anti-inflammatory state seems to be crucial for tissue regeneration. During this process, specific cell types recruit and attract others to the injury site, facilitating tissue renewal and adaptation (193). As a result, the tissue may become more resilient to subsequent (exercise) bouts.

Another essential finding mainly contributed by paper 3 (meta-analysis) exhibits potential tendon injury preventive properties of collagen peptides indicated by an improved tendon morphology (enlarged cross-sectional area). A review on tendon injury reported that Achilles tendon injuries cover almost half of all sports-associated tendon injuries and sport activities appear to be a typical cause of Achilles tendon ruptures (194). Although less common than Achilles tendon injuries, Patella tendon injuries - particularly ruptures - frequently occur after trauma, leading to significant impairments in knee extension, gait, and long-term function (195). The number of included studies in paper 3 covering measurements of both tendons was low; however, tendon repair relies on molecular pathways that have been recently reported in collagen peptide administrating trials. Transforming growth factor (TGF)-β and fibroblast growth factor (FGF), both known to stimulate collagen expression and synthesis in tendons in youth and adults, act via TGF-β - Smad2/3 and FGF - ERK/MAPK intracellular signal transduction in mouse tendon maturation (196). Results of in-vitro studies revealed that collagen peptide treatment promoted cell repair and proliferation and collagen synthesis via TGF-β/Smad pathway, at least in skin fibroblasts (197–200). Elevated expression of FGF during wound healing was also apparent after collagen peptide intake (201, 202). Based on these reports, collagen peptides are believed to unfold their cellular repair effects in tendinous tissue. However, to validate such hypothesis, future research should focus on directly investigating the extent of the aforementioned molecular pathways within tendons.

Paper 3 (meta-analysis) illustrated significant reductions in fat-free mass together with enlargements in muscle dimensions (thickness, cross-sectional area and volume). Lean body mass is positively associated with strength capacity and balance (203) whereas low muscle mass is linked to increased surgical and post-operative complications, extended hospital stays, reduced physical function, diminished quality of life, decreased survival rates and increased health care costs (204). Sarcopenia as the age-related muscle atrophy/ loss is further characterized by weakness, decreased autonomy, glucose tolerance and quality of life and increases in inflammation, metabolic syndrome, infiltration of adipose tissue in muscles (205) and overall obesity (206). From a public health perspective, prolonged collagen peptide intake may aid as a beneficial adjunct together with regular exercise to effectively address these widespread societal issues. A recent meta-analysis found that elderly individuals suffering from sarcopenia consume significantly less protein than their non-sarcopenic peers (207). An increased daily protein intake of 1 g/ kg body weight (bw) per day, above the recommended dietary allowance of 0.8 g/ kg bw per day for adults aged 19–65 years (as per the German Nutrition Society), has been associated with improved physical performance and muscle strength (208). Moreover, a suggested intake of approximately 1.3

g/ kg bw per day may further promote and maintain skeletal muscle mass in aging individuals (209). Since achieving such a high protein intake in the elderly can be challenging (210) - partly due to increased susceptibility to chewing difficulties (211) and decreased appetite (212) - liquid or powdered collagen peptides could be incorporated into their diet to help attain the health benefits associated with high protein intake during aging.

4.1 Future horizons

The findings of the presented research project offer several implications for future investigations. Collagen peptide ingestion may affect mechanosensitive cells and tissues involved in movement execution, force generation, and transduction by enhancing collagen synthesis. This likely occurs through the abundant provision of specific amino acids and peptides essential for protein synthesis in corresponding tissues. Collagen, being a major component of the ECM, interacts with 24 yet found functionally distinctive bidirectional transmembrane receptors called integrins that mediate the attachment between cells and their surroundings (213). The interaction between collagen and integrins may influence the mechanical properties of tissues and their ability to respond to mechanical stress, which is especially relevant in the context of exercise and tissue repair. Integrin α7β1, yet the only integrin receptor found exclusively in muscle tissue, binds to its corresponding ligand Laminin (214). This ECM-related glycoprotein has been shown upregulated in biopsy samples of the vastus lateralis following three months of combined resistance training and collagen peptide intake (109), providing initial evidence of a potential interaction between collagen peptides and integrins.

The absorption and subsequent incorporation into target tissues of collagen-derived amino acids, di-, tri- and oligopeptides, their molecular weight (distribution) and optimal dosage also open up new avenues of research. As the majority of long-term studies administered 15 g of collagen peptides (109, 119, 122–126, 128, 130, 131, 135) while also 5 g (9, 10) and 30 g (127) demonstrated significant effects, an efficient administration pattern currently remains elusive. For instance, a recent publication provides preliminary evidence that dosage above 15 g, namely 30 g, significantly enhanced collagen synthesis after acute resistance exercise bouts (58). Since not all studies have reported the amino acid profile of the collagen supplements used, and none has specified the included peptides along with their corresponding molecular weights, future trials are strongly encouraged to include this information.

At last, given collagen's poor "reputation" as a low-quality protein, fortification with limited amino acids or combining it with other protein sources, such as whey protein, could be an effective strategy to harness the physiological benefits of multiple proteins, which have already been disclosed in few trials. Co-ingestion of 25 g of whey with 5 g of collagen

protein was able to avoid post-exercise plasma glycine shortage compared to a total absence of collagen protein (215). Another trial depicted a collagen hydrolysate/ milk protein blend to be a viable alternative to milk protein for older adults, providing a convenient means of supplementing calories and protein to enhance patient adherence and reduce muscle mass loss (89). A mix of 20 g collagen and 25 g whey lead to similar changes in reactive and maximal strength after muscle damaging exercise bouts and three weeks of resistance training as 45 g of pure whey protein (216). Fortifying collagen peptides with only one limited amino acid, namely leucine, failed to reach similar increases in muscle thickness induced by whey protein alone (217). According to these findings, future research could focus on optimizing collagen supplementation strategies by exploring different combinations and fortifications with other protein sources to maximize their physiological benefits. In particular, studies may investigate the ideal ratios of collagen to whey or other proteins to enhance muscle mass retention and recovery, particularly in older adults or individuals undergoing resistance training.

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