Lead Article

The impact of collagen protein ingestion on musculoskeletal connective tissue remodeling: a narrative review

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Collagen is the central structural component of extracellular connective tissue, which provides elastic qualities to tissues. For skeletal muscle, extracellular connective tissue transmits contractile force to the tendons and bones. Connective tissue proteins are in a constant state of remodeling and have been shown to express a high level of plasticity. Dietary-protein ingestion increases muscle protein synthesis rates. High-quality, rapidly digestible proteins are generally considered the preferred protein source to maximally stimulate myofibrillar (contractile) protein synthesis rates. In contrast, recent evidence demonstrates that protein ingestion does not increase muscle connective tissue protein synthesis. The absence of an increase in muscle connective tissue protein synthesis after protein ingestion may be explained by insufficient provision of glycine and/or proline. Dietary collagen contains large amounts of glycine and proline and, therefore, has been proposed to provide the precursors required to facilitate connective tissue protein synthesis. This literature review provides a comprehensive evaluation of the current knowledge on the proposed benefits of dietary collagen consumption to stimulate connective tissue remodeling to improve health and functional performance.

INTRODUCTION

Skeletal muscle tissue is in a constant state of remodeling, with mixed muscle protein turnover rates of between 1% and 2% per day.[1] Physical activity and food ingestion are the main anabolic stimuli for muscle tissue. Dietary-protein ingestion leads to a rapid increase in plasma amino acid concentrations, thereby increasing mixed muscle protein synthesis rates by 40%–50%.[2] A single bout of exercise sensitizes skeletal muscle tissue to the anabolic properties of ingested protein. When combined, exercise and protein ingestion can increase muscle protein synthesis rates by as much as 100%.[3,4] The combined effects of exercise and sufficient dietary-protein provision support muscle conditioning, allowing greater gains in muscle mass and strength after prolonged resistance-type exercise training.[5–7] Over the past decade, evidence has indicated that rapidly digestible, high-quality protein sources (ie, those containing high essential amino acid concentrations, such as dairy and other animal-derived proteins) are effective in stimulating postprandial mixed muscle protein synthesis and can facilitate training-induced increases in muscle mass and strength.[6,8,9]

The generation and transmission of force from muscle tissue to the bone involves several factors, including the muscle extracellular matrix (ECM). The collagenous tissues of the ECM within skeletal muscle have an

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Key words: collagen, hydrolysate, muscle, peptides, protein.

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doi: 10.1093/nutrit/nuab083

important functional role as they provide tissue elasticity and transmit contractile force from myofibrillar proteins in skeletal muscle fibers toward the tendons, ligaments, and bones. Connective tissue function is largely determined by collagen content and cross-linking between collagen fibers. Collagen originally was thought to be inert and resistant to remodeling.[10] However, more recent evidence has proven that collagen and connective tissue protein networks in various musculoskeletal tissues are in a constant state of remodeling.[11,12] Collagen remodeling is regulated by collagen protein synthesis, collagen protein breakdown, and cross-linking activity (enzymatic and nonenzymatic). Physical activity potently increases connective tissue synthesis rates, leading to enhanced remodeling and more effective transfer of contractile force.[13–16] On the other hand, physical inactivity reduces connective tissue synthesis rates.[17,18] Impairments in connective tissue remodeling may lead to structural changes in tissues that compromise their mechanical properties and contribute to the development of musculoskeletal injury. For example, senescent muscle contains more collagen and greater cross-linking,[19] which contribute to greater muscle stiffness.[20] Stiffer connective tissues impair muscle-fiber contractility,[21] compromise force transmission,[22,23] and reduce muscle strength.[24]

The impact of dietary-protein ingestion on connective tissue remodeling remains to be fully defined. Recent studies demonstrated that the ingestion of free essential amino acids or high-quality, rapidly digestible protein sources (ie, whey or casein) does not increase muscle connective tissue protein synthesis rates in young (18–35 y) or older individuals (>65 y).[11,25–28] However, there is a discrepancy between the amino acid profiles of the provided high-quality proteins (ie, low glycine and proline) (Table 1)[29,30] and the amino acid profile of muscle connective tissue (ie, high proline and glycine) (Table 2).[31] Insufficient delivery of these amino acid precursors may prevent an increase in connective tissue protein synthesis. Collagen-derived protein sources (eg, gelatin and collagen peptides) contain substantially greater amounts of proline and glycine (Table 3).[32,33] Considering the high proline and glycine contents of connective tissues, it has been proposed that these collagen-derived protein sources may have the capacity to stimulate and/or support connective tissue protein synthesis rates and promote connective tissue remodeling. However, so far, there seems to be very little evidence to support the anabolic properties of collagen-derived protein sources, despite consistent claims being made in the popular media.

A more complete understanding of collagen network function and its consequences is required to identify potential strategies to improve collagen structure in musculoskeletal tissues. In this review, we provide an overview of the existing literature on collagen structure and function within muscle tissue and focus on the question of whether dietary protein, and collagen protein in particular, could play a role in the conditioning of muscle connective tissue.

Table 1 **Amino acid composition of different animal-based protein sources**[a]

Amino acid	Milk[b]	Casein[b]	Whey[b]	Egg[b]	Beef[c]
Alanine	2.6	2.0	4.2	2.6	1.2
Arginine	2.6	2.1	1.7	2.6	1.2
Cysteine	0.2	0.1	0.8	0.4	0.2
Glutamic acid	16.7	13.9	15.5	5.1	2.8
Glycine	1.5	1.2	1.5	1.4	1.3
Histidine	1.9	1.7	1.4	0.9	0.6
Isoleucine	2.9	2.3	3.8	1.6	0.8
Leucine	7.0	5.8	8.6	3.6	1.5
Lysine	5.9	4.6	7.1	2.7	1.5
Methionine	2.1	1.6	1.8	1.4	0.5
Phenylalanine	3.5	3.1	2.5	2.3	0.7
Proline	7.3	6.5	4.8	1.8	0.9
Serine	4.0	3.4	4.0	3.3	0.7
Threonine	3.5	2.6	5.4	2.0	0.7
Tyrosine	3.8	3.4	2.4	1.8	0.5
Valine	3.6	3.0	3.5	2.0	0.9

[a]Values presented are in grams of amino acids per 100 g of raw material. Tryptophan, aspartic acid, asparagine, and glutamine were not measured.
[b]Data extracted from Gorissen et al.[29]
[c]Raw lean ground beef:, 85% lean meat, 15% fat (18.6 g protein).[30]

Table 2 **Amino acid composition of collagen subtype α chains**[a]

Amino acid	α1(I)	α2(I)	α1(II)	α1(III)	α1(IV)	α2(IV)
Alanine	115	102	103	96	30	47
Arginine	50	50	50	46	22	42
Aspartic acid	42	44	43	42	45	49
Glutamic acid	73	68	89	71	78	65
Glycine	333	338	333	350	334	324
Cysteine	0	0	0	2	0	2
Histidine	3	12	2	6	6	6
Hydroxylysine	9	12	20	5	50	36
Hydroxyproline	109	94	99	125	123	111
Leucine	19	30	26	22	52	56
Lysine	26	18	15	30	6	7
Methionine	7	5	10	8	15	14
Phenylalanine	12	12	13	8	27	36
Proline	124	113	120	107	85	73
Serine	34	30	25	39	38	30
Threonine	16	19	23	13	19	30
Tyrosine	1	4	2	3	5	7
Valine	21	35	18	14	33	27

[a]Values are number of amino acids per 1000 amino acids.[31]

COLLAGEN STRUCTURE AND REMODELING

Collagen structure

Collagen primarily acts as an integral structural component of the ECM within various tissues. Collagen is ubiquitous within various tissues, constituting approximately 25%–30% of all body protein. At least 28 different types of collagen proteins have been identified.[34,35]

Table 3 **Amino acid composition of different sources of collagen**[a]

Amino acid	Ox hide gelatin[b]	Commercial bone gelatin[b]	Pig skin gelatin[b]	Whaleskin gelatine[b]	Ox-bone gelatin[b]	Human bone collagen[b]	Human tendon acid extract[b]	Wallaby tendon[b]	Sturgeon swim-bladder collagen[c]	Cod-bone gelatin[c]	Shark skin gelatin[c]	Lung-fish skin collagen[c]	Lung-fish skin gelatin[c]
Alanine	11.0	11.3	10.7	10.8	10.5	10.9	10.3	10.7	11.6	10.4	11.2	11.7	11.9
Arginine	8.8	9.0	9.1	9.5	9.2	8.8	8.9	9.5	10.0	9.1	9.3	9.1	9.9
Aspartic acid	6.7	6.7	6.7	6.7	7.1	6.7	6.7	7.0	6.9	7.5	6.0	6.6	6.2
Cystine	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glutamic acid	11.4	11.6	11.3	11.2	11.9	11.4	11.1	11.5	11.4	11.4	10.3	11.9	11.9
Glycine	27.5	27.2	26.4	26.7	25.3	25.8	25.4	25.7	27.7	28.2	26.5	24.0	26.1
Histidine	0.8	0.7	1.0	1.0	1.0	1.0	0.9	0.8	0.8	1.2	1.3	0.8	0.8
Hydroxylysine	1.0	0.8	1.0	1.0	1.1	0.6	1.5	1.4	1.9	1.4	0.8	0.9	1.1
Hydroxyproline	14.1	13.3	13.5	12.8	14.1	14.1	12.6	13.0	11.8	8.3	10.9	9.8	10.8
Isoleucine	1.7	1.5	1.4	1.6	1.7	1.9	1.5	1.3	1.7	1.6	2.7	1.6	1.3
Leucine	3.3	3.5	3.3	3.6	3.9	3.6	3.5	3.7	2.6	3.3	3.3	3.4	2.8
Lysine	4.5	4.4	4.1	4.1	4.1	4.4	3.3	3.8	3.5	3.7	3.8	3.6	3.6
Methionine	0.9	0.6	0.9	0.8	0.8	0.8	0.9	1.1	1.4	2.3	1.6	0.6	0.5
Phenylalanine	2.2	2.5	2.6	2.3	2.9	2.5	2.5	2.8	2.5	2.0	2.4	2.6	2.4
Proline	16.4	15.5	16.2	16.2	14.7	15.3	15.2	14.7	12.8	12.4	13.9	14.8	15.8
Serine	4.2	3.7	4.1	4.7	4.2	4.1	4.1	4.4	5.8	7.9	5.0	4.7	4.7
Threonine	2.2	2.4	2.2	3.1	2.5	2.4	2.3	2.6	3.8	3.2	3.2	3.2	3.0
Tyrosine	0.3	0.2	0.6	0.7	0.6	0.9	0.7	0.8	0.5	0.6	0.3	0.2	0.1
Valine	2.6	2.8	2.8	2.6	2.7	3.0	3.1	2.9	2.3	2.3	2.7	2.6	2.2

[a]Values presented are in grams of amino acids per 100 g of protein.
[b]Data extracted from Eastoe.[32]
[c]Data extracted from Eastoe.[33]

Table 4 **Collagen subtypes, function, and anatomic location**[a]

Molecular type	Key aspects	Constitution	Synthesizing cells	Function	Location (tissue)
I (Fibril forming)	Most abundant collagen type	2 α1(I) chains, 1 α2(I) chain	Fibroblasts, osteoblasts, odontoblasts, cementoblasts	Resists tension	Dermis, tendon, ligaments, capsules of organs, bone, dentin, cementum
II (Fibril forming)		3 α1(II) chains	Chondroblasts	Resists tension	Hyaline cartilage, elastic cartilage
III (Fibril forming)	Highly glycosylated, known as reticular fibers	3 α1(III) chains	Fibroblasts, reticular cells, smooth-muscle cells, hepatocytes	Forms structural framework of spleen, liver, lymph nodes, smooth muscle, adipose tissue	Lymphatic system, spleen, liver, cardiovascular system, lung, skin
IV (Network forming)		2 α1(IV) chains, 1 α2(IV) chain	Epithelial cells, muscle cells, Schwann cells	Forms meshwork of the lamina densa of the basal lamina to provide support and filtration	Basal lamina
V (Fibril forming)		2 α1(V) chains, 1 α2(V) chain	Fibroblasts, mesenchymal cells	Associated with type I collagen and with placental ground substance	Dermis, tendon, ligaments, capsules of organs, bone, cementum, placenta

[a]Data extracted from Gartner.[37]

However, collagen types I, II, III, and IV are most abundant in musculoskeletal tissues.[36]

All collagen types share a similar right-handed, triple-helix sequence consisting of 3 α chains of peptides, which can be homogenous (eg, type II: 3 α-1 chains) or heterogenous (eg, type I: 2 α-1 chains and 1 α-2 chain) in composition (Table 4).[37] The α chains contain approximately 1000 amino acid residues arranged in the repeating peptide sequence Gly-X-Y. Therefore, glycine occupies every third position along the α chain. The X and Y positions can be occupied by any amino acid, although most frequently they are occupied by proline and hydroxyproline, respectively. Because of the repeating peptide sequence, collagen proteins contain high concentrations of glycine (∼33%), proline (∼10%), and hydroxyproline (∼13.5%) relative to other proteins. Glycine has a small molecular footprint, which adds stability to the α chain and permits close orientation with other α chains. The amino acid structures of proline and hydroxyproline allow twisting of the α chain, and the hydroxyl group of hydroxyproline stabilizes the triple helix structure at body temperature. The hydroxylated form of lysine (ie, hydroxylysine) is also characteristically found in collagen α chains and permits the formation of collagen fibrils by binding α chains to each other through cross-linking (described in the section Enzymatic cross-linking). Slight differences in amino acid composition exist between the different α chains and, thus, collagen types (Table 2). However, all collagen types are similar in that they contain very low amounts of cysteine and no tryptophan.

Collagen synthesis

A graphical representation of collagen synthesis is presented in Figure 1. Collagen turnover is estimated to occur at a rate between 0.5% and 2% per day, which is within a similar range as myofibrillar proteins.[1,11,12,15,38,39] This rate of collagen turnover translates to a half-life of approximately 2–5 months. Collagen synthesis originates in fibroblasts, which are embedded within the ECM of various tissues. Active fibroblasts are near collagen fibers, lying in a parallel orientation along the longitudinal axis of the collagen fibers.[40] Collagen synthesis begins intracellularly with the assembly of the precursor to mature collagen, termed *procollagen*. The assembly of procollagen occurs primarily in the rough endoplasmic reticulum.

Translation is followed by multiple stages of post-translational modification including: 1) post-translational hydroxylation of lysine and proline on monomer α chains into hydroxylysine and hydroxyproline, respectively; 2) subsequent, additional post-translational glycosylation of hydroxylysine; and 3) aggregation of monomer α-collagen chains through cross-linking into a triple-helix procollagen structure. Both ends of the procollagen structure contain propeptide regions that contain disulfide bonds between α chains to stabilize the region. For type I procollagen, these regions are termed procollagen type I N-terminal propeptide and procollagen type I carboxy-terminal propeptide for the carboxyl terminal, respectively. Once formed, the procollagen structures are transported to the Golgi apparatus, where they are packaged into vesicles and transported out of the fibroblast. Extracellularly, procollagen peptidases

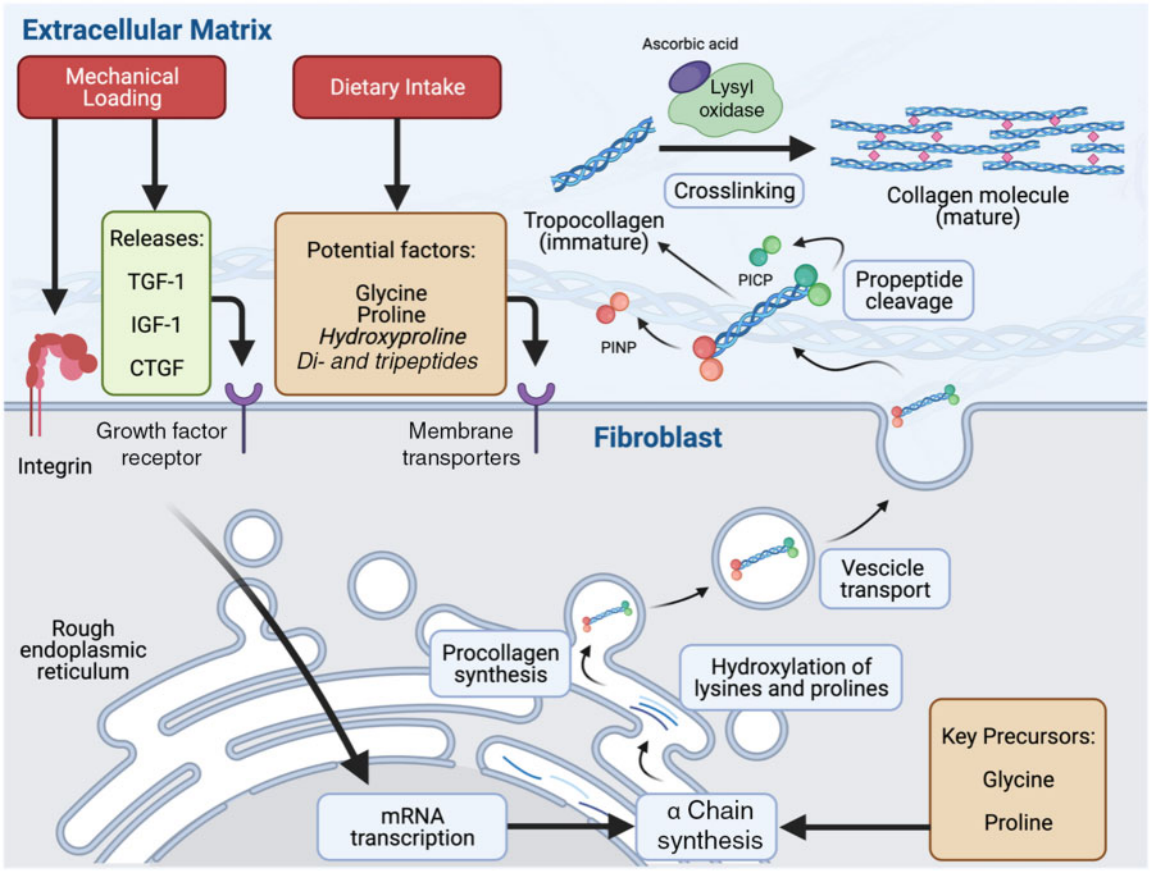

Figure 1 **Graphical representation of the key stages in collagen synthesis.** Fibroblasts are embedded in a longitudinal orientation within the extracellular matrix (ECM) of various tissues (eg, skeletal muscle, tendon). Mechanical loading results in increased tension within the connective tissue, resulting in signal transduction through integrin activation and/or the release and binding of growth factors (eg, TGF-1, IGF-1, CTGF) on their respective membrane receptors. These signals are transduced to the nuclei of the fibroblast, resulting in increased mRNA transcription and α chain synthesis, which occurs in the rough endoplasmic reticulum. Part of the prolines and lysines in the α chains are hydroxylated into hydroxyproline and lysine, respectively, and 3 α chains are assembled into procollagen. Procollagen is packaged into vesicles and exported to the ECM. Extracellularly, procollagen peptidases cleave the propeptide regions (eg, procollagen type I N-terminal propeptide, and procollagen type I carboxy-terminal propeptide), allowing the resulting collagen monomers (tropocollagen) to stack spontaneously in an overlapping and parallel fashion. Lysyl oxidase activity, which requires ascorbic acid as a cofactor, forms cross-links between tropocollagen molecules, resulting in the formation of mature collagen within the extracellular network. Food ingestion, and collagen peptides in particular, has been proposed to deliver anabolic stimuli (eg, hydroxyproline, peptides) and key precursor amino acids (eg, glycine and proline) required to increase collagen synthesis. However, the proposed anabolic properties of food intake on muscle connective tissue synthesis rates in humans appear to have no stimulatory role (eg, leucine-rich protein) or have not yet been examined (eg, hydroxyproline, glycine, proline, peptides). CTGF, connective tissue growth factor; IGF-1, insulin-like growth factor-1; TGF-1, transforming growth factor-1

cleave the procollagen type I N-terminal propeptide and procollagen type I carboxy-terminal propeptide regions, allowing the resulting collagen monomers, termed *tropocollagen molecules*, to stack spontaneously in an overlapping and parallel fashion.

Enzymatic cross-linking

The formation of covalent bonds between lysine and hydroxylysine residues of neighboring tropocollagen molecules facilitates formation of mature collagen fibrils. The formation of these so-called cross-links is enzymatically catalyzed by lysyl oxidase, which forms pyridinoline between (hydroxy)lysine residues of adjacent collagen fibrils. The positioning of pyridinoline cross-links facilitates a staggered arrangement of collagen fibrils, each overlapping another by one-third to provide strength to the entire collagen molecule. The staggered configuration, along with a greater abundance of cross-links within collagen fibrils, results in greater tissue stiffness. The degree of cross-linking relative to collagen abundance varies among different tissues and generally relates to the functional properties of the tissue. For example, the pyridinoline-to-collagen ratio is especially high in tendon, compared with bone.[41] In animal models, inhibition of lysyl oxidase, and therefore a reduction in collagen cross-linking, results in collagen fibrils and tendons with reduced strength.[42]

Nonenzymatic cross-linking

A separate form of cross-links, known as advanced glycation end products, exist in connective tissues. The accumulation of advanced glycation end products is the result of the long-lived nature (ie, slow turnover) of mature collagen and prolonged exposure to monosaccharides. With prolonged exposure, spontaneous nonenzymatic bonds are formed between a reducing sugar and a protein residue on mature collagen (eg, lysine side chains). The development of nonenzymatic cross-links (eg, pentosidine, glucosepane) follows a complex series of reactions, known as the Maillard reaction, over the course of months or years.[43] The presence of advanced glycation end products increases with increased plasma glucose concentrations (eg, diabetes) and with advanced aging.[19,43–46] Similar to enzymatic cross-linking, nonenzymatic cross-linking increases the strength and stiffness of the connective tissue.[20,47,48] Although this leads to greater failure loads, the uncontrolled nature of nonenzymatic cross-linking can be problematic, because overly stiff connective tissues are more prone to injury (eg, tissue rupture)[49] and limit tissue compliance. Low compliance of the ECM restricts radial expansion of contracting muscle, limiting the force-generating capacity of muscle.[21]

Collagen degradation

Collagen degradation follows a 3-step process that occurs extracellularly, primarily by matrix metalloproteinases (MMPs). In the first step, MMPs are activated and bind to collagen fibrils. In the second step, the bound MMPs unwind the triple-helix structure to allow better access to the individual α strands. In the last step, MMPs cleave the individual strands at predictable regions. In collagen types I, II, and III, cleavage occurs at specific glycine-isoleucine bonds of the α-1 chains and a specific glycine-leucine bond of the α-2 chain.[50–55] Cleavage at these bonds generates characteristic N-terminal and C-terminal fragments that are, respectively, three-fourths and one-fourth the length of the original α-chains.[50] The fragments are unstable at body temperature, resulting in denaturation before further degradation by gelatinases (MMP-2, MMP-9) or other nonspecific proteases.[56]

The activation of MMPs is regulated by 1) gene transcription; 2) disruption of the thiol-Zn^{2+} interaction, which exposes the catalytic binding region; and 3) interaction with inhibitors such as the tissue inhibitors of metalloproteinases. There is substantial overlap in the substrate specificity of different members of the MMP family.[57] However, MMP activity is selectively targeted toward the intended substrate through differences in enzyme affinity and compartmentalization. Compartmentalization of MMPs is achieved by the presence of a molecular component (eg, anchoring to cell membranes), which restricts the MMP activity to the immediate environment. MMP-1 (collagenase-1), MMP-8 (collagenase-2), MMP-13 (collagenase-3), MMP-18 (collagenase-4), MMP-2 (gelatinase-A), and MMP-14 (MT1-MMP) can cleave collagen types I, II, and III[58] and are, therefore, most relevant for musculoskeletal tissues.

THE IMPACT OF PHYSICAL ACTIVITY ON COLLAGEN REMODELING

Mechanical loading as a main stimulus

Mechanical loading has been shown in vitro to stimulate fibroblasts to increase collagen synthesis over an acute period.[59] In connective tissues, fibroblasts are generally arranged longitudinally along the collagen fibers. The fibroblasts are typically bound to the ECM by way of membrane-bound integrins. This configuration exposes fibroblasts to the forces transmitted though the muscle and the ECM, in particular. Fibroblasts sense the mechanical load and upregulate molecular processes (ie, synthesis) to facilitate appropriate tissue remodeling.[59] In particular, it has been suggested that mechanical loading stimulates the release of growth factors (eg, transforming growth factor-1, connective tissue growth factor, and insulin-like growth factor-1), some of which (namely, transforming growth factor-1, connective tissue growth factor) act directly on fibroblasts to upregulate collagen synthesis.[60–64] The anabolic impact of these growth factors on collagen synthesis has been demonstrated in various overload models and in response to a bout of treadmill running in rodents.[63,65–67] However, it remains unclear whether these findings translate to humans, because growth factor expression has been shown to increase,[68] remain unchanged,[69,70] or even decrease[69,70] in tendon tissue after exercise. Discrepancies between these findings may be due to differences in exercise duration and/or intensity, or differences in tissue sampling location. Mechanical loading has also been shown to upregulate transcriptional activity of lysyl oxidase after resistance-type exercise training in rats, suggesting that prolonged training may lead to greater cross-linking and stiffening of connective tissue.[65]

Exercise-induced adaptations in intramuscular connective tissue

Both endurance-[71,72] and resistance-type exercise[13,14,25,27,28,73] increase muscle connective tissue protein synthesis in humans. Lengthening contractions

more robustly increase postexercise intramuscular connective tissue protein synthesis rates than do shortening contractions, reflecting the potent stimulatory effect of increased tension within contracting muscle tissue.[39,74] The impact of prolonged exercise training on intramuscular connective tissue protein content has not been well explored in humans, though animal work has suggested that prolonged exercise training increases intramuscular connective tissue protein (ie, collagen) contents.[75–77] Overall, exercise training–induced increases in collagen content and the level of cross-linking are likely critical to allow transmission of greater contractile forces from the muscle to tendon, ligament, and bone. In support, findings from recent work demonstrated that enhanced ECM remodeling is associated with increases in muscle mass and strength in overloaded rodent plantaris muscle.[16] More exploration is required to determine whether the association between enhanced ECM remodeling and increases in muscle mass and strength following resistance-type exercise training in humans is both real and causal.

THE IMPACT OF DIETARY PROTEIN ON COLLAGEN REMODELING

Dietary-protein ingestion to support (muscle) tissue adaptation

It is well known that food ingestion is one of the most potent stimuli that promotes and/or supports musculoskeletal tissue adaptation. In particular, the ingestion of dietary protein potently increases muscle protein synthesis rates.[38] The postprandial increase in plasma (essential) amino acids is a key factor for driving the increase in postprandial muscle protein synthesis rates.[78] This anabolic response seems to be largely driven by the postprandial increase in circulating leucine levels and is further supported by the ample availability of amino acids as precursors for de novo muscle protein synthesis. In particular, leucine co-ingested with a suboptimal dose of protein further stimulates an increase in muscle protein synthesis rates at rest[79–83] and during postexercise recovery.[79–82,84,85] However, the increased muscle protein synthesis rates cannot be sustained without the delivery of other amino acids to serve as precursors.[86] Together, these findings illustrate that the ingestion of dietary protein not only provides the anabolic signal to upregulate muscle protein synthesis (ie, leucine) but also delivers the amino acid precursors that are required to sustain muscle protein synthesis rates.

The properties of nutrition, and dietary protein in particular, that may specifically enhance connective tissue adaptation in the musculoskeletal tissues have not been fully elucidated. Seminal work by Babraj et al[11] demonstrated that the ingestion of a 20-g mixture of essential amino acids did not increase intramuscular collagen protein synthesis rates in young and older individuals nor did it increase tendon collagen synthesis rates in young individuals. The absence of an impact of amino acid administration to enhance connective tissue protein remodeling was corroborated by Mikkelsen et al[28] and Dideriksen et al,[25] who demonstrated that the ingestion of 20–38 g of whey protein did not increase intramuscular collagen protein synthesis rates in older individuals at rest. Recently, we demonstrated that the ingestion of a larger, 40-g dose of casein protein does not result in increase in intramuscular collagen protein synthesis rates over a more prolonged postprandial period during overnight sleep (7.5 h).[13]

Dietary-protein ingestion and postexercise connective tissue protein synthesis rates

Although the combination of exercise and dietary-protein ingestion is well known to have an additive effect on stimulating muscle protein synthesis rates, dietary-protein intake does not appear to further increase the postexercise increase in connective tissue protein synthesis rates. For example, Holm et al[27] showed that a pulse protein-feeding pattern (2–3 g of a soy or milk protein mixture ingested every 30 min) does not further increase postexercise intramuscular collagen protein synthesis rates during the early (ie, 30 min to 3 h) or late (3–5.5 h) phase of recovery from a bout of high- or low-intensity resistance-type exercise. More recent work by Holm et al[74] suggested that the ingestion of an ~18 g bolus of whey protein further increased intramuscular connective tissue protein synthesis rates during the later phase (2–5 h) of postexercise recovery when compared with the ingestion of carbohydrate. This finding suggests that protein ingestion may have a more delayed impact on increasing intramuscular collagen protein synthesis rates. However, recent work by Trommelen et al[14] contests this hypothesis by demonstrating that the ingestion of a larger bolus of high-quality protein (30 g of casein) does not further increase postexercise intramuscular collagen protein synthesis rates throughout the subsequent 7.5 h of overnight sleep.

Although dietary-protein ingestion does not appear to stimulate connective tissue protein synthesis rates, the recent application of intrinsically labeled foods has indicated that protein ingestion delivers dietary-derived amino acids for incorporation in connective tissue proteins. The ingestion of specifically produced highly L-[1-^{13}C]-phenylalanine–enriched (> 35 mole percent excess) casein protein combined with tissue sampling

allows for the assessment of the metabolic fate of dietary protein–derived amino acids into newly synthesized proteins, termed de novo protein synthesis.[87,88] Using this approach, Trommelen et al[14] demonstrated for the first time that dietary protein–derived amino acids are, indeed, incorporated into de novo intramuscular connective tissue protein. Follow-up work demonstrated that the utilization of the ingested protein-derived amino acids for de novo intramuscular connective tissue protein synthesis is further enhanced during recovery from resistance exercise.[13] Together, the observations that dietary protein–derived amino acids are incorporated into connective tissue protein and modulated with physical activity implies that dietary provision of amino acids is required and that there may be conditions where intramuscular connective tissue remodeling can be facilitated or compromised. For instance, it has been speculated that protein sources delivering large amounts of the key amino acids used for connective tissue protein synthesis (ie, glycine and proline) may be more suitable for facilitating increased connective tissue protein synthesis rates.[13,14,89,90] This hypothesis is discussed in the following sections.

Dietary collagen ingestion to enhance connective tissue remodeling

Because the ingestion of essential amino acids, whey, or casein does not appear to stimulate intramuscular connective tissue protein synthesis rates, dietary-protein sources with alternative properties have been proposed to be more suitable to promote intramuscular connective tissue protein remodeling. For instance, rodent and ex vivo tissue engineering models have indicated that the provision of free glycine and proline promotes an increase in collagen synthesis.[91,92] As such, dietary-protein sources that are rich in glycine and proline may be more suitable than dairy protein to promote tissue collagen synthesis. Collagen-derived dietary-protein sources, such as gelatin and collagen hydrolysate, provide large amounts of proline and glycine (Table 3). It has been proposed, therefore, that collagen-derived protein sources may be preferred to support connective tissue protein remodeling.[89] At present, however, the digestion and absorption properties of collagen-derived dietary proteins and the biological impact on connective tissue remodeling in various human tissues remain to be elucidated.

Collagen extraction and industrial processing for human consumption

A graphical representation of collagen extraction and industrial processing is presented in Figure 2. Collagen-derived dietary proteins are most commonly extracted from porcine skin (45%), bovine hide (30%), and the bones of both animals (23%).[93] Chicken (bones) and fish (scales and bones) byproducts are also used as production sources of collagen-derived dietary protein. Collagen derived from fish sources have generally been shown to contain lower proline and hydroxyproline contents than collagen derived from pig or bovine sources, though warm-water fish tend to contain the highest proline and hydroxyproline contents among the different species.[94,95] The process of collagen extraction varies slightly depending on the matrix of the collagen source (eg, bone has a high mineral content, whereas porcine skin has a high fat content). In general, however, collagen extraction involves trimming and cleaning of the source material; pretreatment to remove minerals, fats, or other noncollagen material; extraction; filtration; concentration; sterilization; and drying. The extraction of gelatin requires exposure to high temperatures (50–100°C), which denature the triple-helix structure of collagen and result in more soluble peptide strands that are lower in molecular weight (~100 kDa) than intact collagen (300 kDa). The extraction of a more intact form of collagen is achieved when the source material is exposed to various acids at lower temperatures (2–8°C).[95]

Extracted collagen and gelatin can be further processed using additional acid-exposure steps or enzymatic hydrolysis to produce collagen hydrolysates. Depending on the collagen source and hydrolyzation protocol, collagen hydrolysates can vary in size, with molecular weights ranging from 2 to 20 kDa, though most commonly < 6 kDa.[96,97] Collagen hydrolysates of different molecular weights possess different properties, which are relevant when used as ingredients in food preparation (eg, emulsifiers, stabilizers, and enhancers that bind other ingredients). The processing of collagen may also have significant bearing on the digestion and absorption kinetics of collagen-derived dietary proteins and their subsequent biological impact on connective tissue remodeling in various human tissues (eg, skeletal muscle, tendons, ligaments, bones, and skin).[98,99]when used

Digestion and absorption of collagen-derived dietary proteins

Native collagen is resistant to peptide cleavage by digestive enzymes, resulting in poor absorption (as low as ~10%) into the circulation.[100] Production into gelatin increases digestion and absorption, though perhaps not to the extent of other dietary proteins. For instance, work using rodent models has shown that relatively more nitrogen is recovered from the intestine of rats

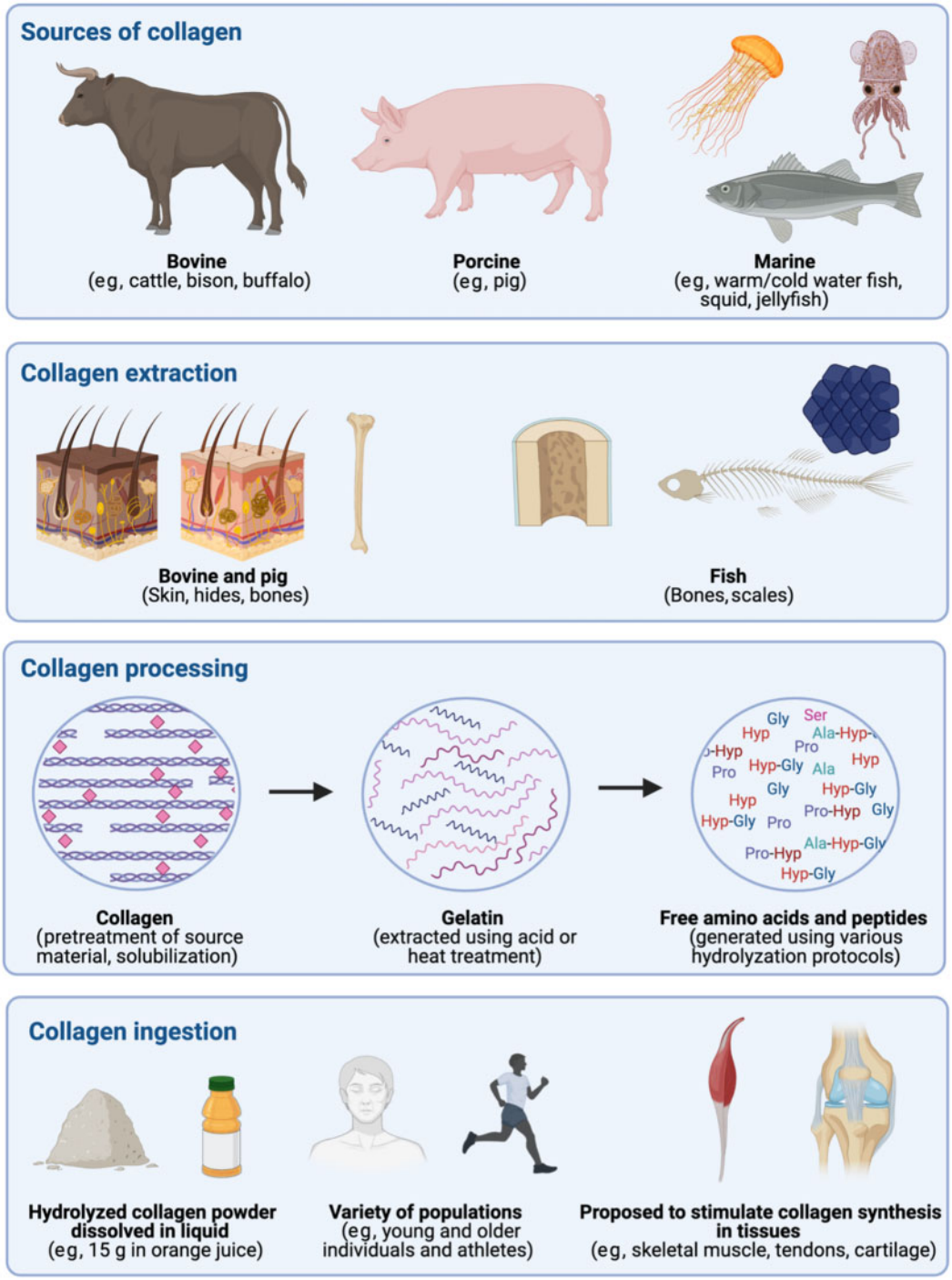

Figure 2 **Graphical representation of the industrial sources of dietary collagen, the extraction procedures used to produce hydrolyzed collagen, and the proposed applications of collagen peptide ingestion in humans.** The currently established industrial sources of collagen are of bovine (eg, cattle, bison buffalo), porcine (ie, pig), and marine (eg, warm- and cold-water fish, squid, jellyfish) origin. Collagen source materials from these animals include skin, bones, and scales, which are considered byproducts generated from processing for other purposes (eg, food production). The source materials undergo pretreatment, including cleaning, hair removal, and collagen solubilization using chemical treatment. Collagen is further extracted using acid or heat treatment, which results in gelatin release. Gelatin consists of denatured and unbound collagen fibers. Gelatin may undergo various hydrolyzation processes that result in the liberation of free amino acids and peptides, which may resist complete hydrolyzation. It has been suggested that certain peptides have stimulatory or inhibitory properties, though, to our knowledge, no human in vivo evidence has been generated to support such claims. Hydrolyzed collagen is soluble in liquid and, therefore, is more readily available for application as a dietary supplement (eg, 15 g of collagen hydrolysate dissolved in orange juice). Current claims for the benefits of collagen supplementation include application in younger (18–35 y) and older (>65 y) individuals and athletes to enhance collagen remodeling in skeletal muscle, tendons, ligaments, cartilage, and skin.

fed gelatin in comparison with casein or egg albumin.[101] Chen et al[102] built on this finding by measuring intestinal contents and determined that gelatin feeding resulted in longer peptides (6.0 amino acid residues/peptide) than did casein feeding (2.7 amino acid residues/peptide) and a nitrogen-free diet (2.4 amino acid residues/peptide), suggesting less complete gelatin digestion. In humans, Shaw et al[89] demonstrated that the ingestion of 5 g and 15 g of gelatin increases plasma glycine, proline, and hydroxyproline concentrations in comparison with placebo ingestion. Gelatin contains high(er) concentrations of glycine, proline, and hydroxyproline; thus, these findings suggest that gelatin is digested and subsequently absorbed into the circulation. The ingestion of 15 g of gelatin resulted in greater peak plasma concentrations of glycine (~1.75-fold), proline (~1.6-fold), and hydroxyproline (~2-fold) than did the ingestion of 5 g of gelatin. The greater plasma concentrations of these amino acids remained elevated over the 3 -hour postprandial period in comparison with the ingestion of 5 g gelatin. These findings indicate a dose-dependent pattern of gelatin ingestion on peak plasma amino acid concentrations and amino acid availability. Others have reported plasma amino acid concentrations after the ingestion of 35 g of hydrolyzed collagen[103] similar to those reported after the ingestion of 15 g gelatin.[89] The similarity between peak plasma concentrations may suggest that the ingestion of ~15 g dietary collagen-derived protein ingestion results in near-maximal rates of amino acid absorption from gelatin. However, digestion and absorption kinetics after collagen-derived protein ingestion (eg, the relative release into the plasma circulation and splanchnic uptake) remain to be determined in the human in vivo setting.

Digestion and absorption of hydrolyzed collagen

The hydrolyzation of dietary protein into peptides increases the postprandial rate of dietary protein–derived amino acid appearance into the plasma circulation after protein (hydrolysate) ingestion. For instance, Koopman et al[98] demonstrated that hydrolyzed casein ingestion resulted in a greater rate of dietary protein–derived amino acid appearance, resulting in greater plasma amino acid availability and subsequent delivery to tissues, in comparison with the ingestion of intact casein. Oesser et al[104] used rodent models to demonstrate that an orally administered dose of ^{14}C-labelled hydrolyzed gelatin (3–6 kDa) was released to a similar extent (95% of ingested protein) in comparison with a matching oral dose of ^{14}C-labelled free proline over a relatively prolonged 12-hour postprandial period. It has been proposed, therefore, that the hydrolyzation of collagen protein sources may enhance collagen protein digestion and amino acid absorption. However, Alcock et al[90] have recently examined plasma amino acid profiles in human volunteers after the ingestion of either 20 g of gelatin or 20 g of hydrolyzed collagen. The ingestion of both collagen sources resulted in similar peak glycine concentrations, and no differences were observed in total plasma amino acid availability (incremental area under the curve), despite the apparently lower molecular weight of hydrolyzed collagen. Lis and Baar[105] corroborated these findings, demonstrating that the ingestion of 15 g of hydrolyzed collagen and gelatin results in nearly identical postprandial plasma glycine, lysine, proline, hydroxyproline, but not leucine, concentrations at 1 hour after protein ingestion. The absence of differences in postprandial plasma amino acid profiles after the ingestion of gelatin vs hydrolyzed collagen may be due to protein processing. For instance, different hydrolyzation protocols are known to produce peptides of larger molecular weights.[95] Skov et al[103] recently demonstrated that ingestion of 35 g of enzymatically hydrolyzed collagen protein results in greater plasma glycine, proline, and hydroxyproline availability (incremental area under the curve) in comparison with the ingestion of 35 g of nonenzymatically hydrolyzed collagen. Work comparing digestibility after different hydrolyzation protocols of the same source of collagen-derived protein is required to identify characteristics that maximize the uptake of collagen protein-derived amino acids into the circulation.

Potential mechanisms involved in collagen peptide digestion and absorption

It has been proposed that the hydrolyzation of collagen-derived proteins produces peptides, which may act to further enhance connective tissue remodeling in musculoskeletal and dermal tissues.[104,106,107] To have any stimulatory or inhibitory properties, the ingested peptides must be absorbed in their intact form into the circulation and taken up into target tissues. Peptide absorption in the gut may occur through 4 possible pathways: 1) through the tight junctions between enterocytes, 2) by passive diffusion through the enterocytes, 3) by endocytosis through the enterocyte, and 4) through transmembrane transporter protein systems.[108] However, no in vivo evidence has been generated to directly support the transport of peptides through tight junctions, passive diffusion, or endocytosis.[108] With regard to active transport, a central transmembrane peptide transporter, PEPT-1, has been identified and its functionality has been defined (Figure 3). The PEPT-1 transporter is located on the brush border of the intestinal epithelium,[109] and several studies have determined that it facilitates transport of only di- and tripeptides, as

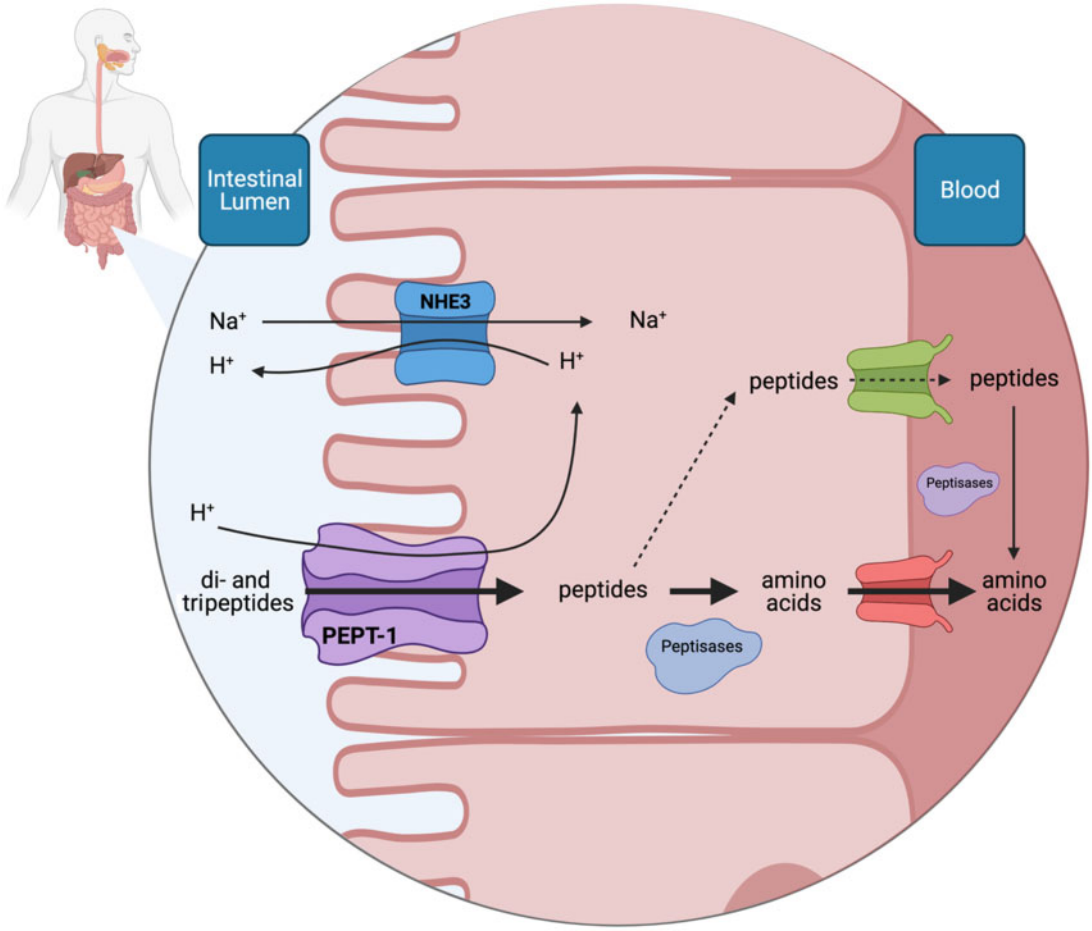

Figure 3 **Graphical representation of collagen-derived di- and tripeptide absorption from the intestinal lumen into the blood circulation.** Various hydrolyzation procedures during industrial processing of collagen produce peptides, which have been proposed to have stimulatory or inhibitory properties within various tissues. Hydrolyzed collagen ingestion delivers peptides to the intestinal lumen. Collagen-derived peptides, and those containing hydroxyproline in particular, appear to be resistant to further intestinal peptide cleavage into free amino acids. These peptides may be transported through a transmembrane peptide transporter (PEPT1) into the enterocyte. Peptides transported to the cytosol of the enterocyte are either cleaved by peptidases into free amino acids and transported further into the circulation or are transported in their intact form into the circulation. Once in the circulation, peptides are subjected to cleavage through exposure to vascular endothelial tissue or peptidase activity. Overall, the existing evidence suggests that intact dietary-derived peptide uptake into the circulation is low. Whether collagen peptides, some of which are proposed to be more resistant to cleavage, have a stimulatory impact on connective tissue remodeling remains to be determined in humans

opposed to free amino acids and peptides longer than 4 amino acids, into the cytosolic space of the enterocyte.[110–112] However, further transport of di- or tripeptides through the enterocyte into the hepatic portal vein is low.[113–115] This is due to the presence of peptidases in the cytosolic space of the enterocyte that further hydrolyze peptides into their amino acid constituents,[116] which are then transported into the hepatic portal vein. In the case that di- and tripeptides are, indeed, transported from the gut into the circulation, they are subjected to further hydrolysis by exposure to vascular endothelial tissue and plasma peptidases.[114,117,118] However, although most (if not all) peptides are hydrolyzed during transport into the circulation, there is some evidence to suggest that specific collagen-derived di- and tripeptides can be absorbed intact, with those containing hydroxyproline and/or proline proposed to resist peptidase activity.[119] In support of this proposition, early work demonstrated that the ingestion of 30 g of gelatin increased hydroxyproline-containing dipeptides, but not free hydroxyproline, in the urine of human participants.[120] These findings were later corroborated by Weiss and Klein,[121] who demonstrated that the administration of ^{14}C-labeled proline-hydroxyproline (Pro-Hyp) in gelatin and in peptide form could be detected in the urine, suggesting intact Pro-Hyp absorption and excretion. Pro-Hyp is the most abundant peptide contained in collagen hydrolysate,[122–124] and has been shown in vitro to be resistive to peptidase action in plasma.[123]

Current evidence regarding dietary collagen peptide absorption into plasma

The proposed absorption of collagen-derived peptides remains poorly described, primarily due to the analytical challenges of producing labeled internal standards for quantifying concentrations of the many different peptides within plasma samples. In early studies, researchers were able to determine that Hyp-containing peptide concentrations increased in the plasma circulation after gelatin ingestion.[123–125] More recently, investigators were able to assess the presence of more-specific peptides in plasma samples. These studies have identified that the ingestion of ~26 g of hydrolyzed collagen derived from fish scales resulted in a peak Pro-Hyp plasma concentrations of 60 μmol/L.[122] After the ingestion of 10 g of hydrolyzed collagen, peak plasma concentration of Pro-Hyp reached ~20 μmol/L at 60 minutes.[126] These data suggest a dose-dependent response of collagen hydrolysate ingestion and subsequent peptide release, which has also been observed more generally by other investigators.[127] Taga et al[126] demonstrated that after the ingestion of 10 g of porcine gelatin, peak Pro-Hyp concentrations were nearly 8-fold greater than the next highest collagen-derived peptide (Hyp-Gly), though 14- and 5-fold lower than peak free Pro and Hyp concentrations, respectively. This indicates that although specific dipeptides may reach the plasma circulation after gelatin ingestion, their concentration is substantially lower in comparison to the postprandial increase in free amino acid availability.

Effect of collagen-derived protein supplementation on tissue remodeling

Protein ingestion can stimulate and/or support tissue adaptation through 1) the provision of a dietary protein–derived anabolic stimulus that upregulates protein synthesis (eg, leucine for myofibrillar protein synthesis)[83,85]; 2) the provision of dietary protein–derived amino acids as precursors for de novo tissue protein synthesis[86]; and/or 3) through the stimulation of more global postprandial responses (eg, insulin release, enhanced blood flow).[128] Shaw et al[89] demonstrated that the plasma milieu after the ingestion of 15 g of gelatin promoted greater collagen synthesis in ex vivo engineered ligament tissue constructs in comparison with the ingestion of 0 g and 5 g of gelatin. To date, however, the impact of collagen supplementation on connective tissue protein synthesis in vivo in humans has been evaluated in only 1 study. Oikawa et al[129] applied deuterated water methodology and demonstrated that collagen peptide supplementation did not further increase intramuscular collagen protein synthesis rates in comparison with the supplementation of an equivalent dose of whey protein during 3 days of free-living conditions (ie, rest) or during 3 days after a single bout of resistance-type exercise in older women. Without a direct comparison of intramuscular collagen synthesis rates with and without collagen supplementation, it remains unclear whether collagen peptide supplementation provided an anabolic stimulus.

Collagen-derived protein supplementation on long-term clinical outcomes

Some evidence exists to support the impact of collagen-derived protein supplementation on the remodeling of various musculoskeletal tissues in healthy and clinical populations. For tendon, researchers found in a recent pilot study that oral collagen peptide supplementation improved symptoms and tendon vascularization in patients with chronic Achilles tendinopathy who were performing a structured exercise program.[130] In bone, König et al[131] recently reported that 12 weeks of collagen peptide supplementation (5 g/d) increased bone mineral density and markers of bone collagen synthesis in postmenopausal women. However, these findings are in partial contrast to those of Cúneo et al,[132] who reported no impact of 24 weeks of collagen peptide supplementation (10 g/d) on comparable markers primarily reflecting bone metabolism in postmenopausal women. The discrepancy between these findings may be related to the nutritional status of the women, differences in the plasma markers measured, and/or production differences of the collagen provided in the supplements. Clifford et al[133] reported no impact of collagen peptide supplementation (20 g/d) on circulating markers of bone metabolism (procollagen type I N-terminal propeptide, CTX-I) after a single bout of exercise.

Although the role of collagen supplementation on bone remodeling remains unclear, it is noteworthy to highlight that early stable isotope work indicated that intravenous infusion of lipids, glucose, and free amino acids stimulates an increase in bone collagen synthesis in humans.[134] These data suggest a potential role of nutritional interventions to enhance bone remodeling. There is clearly a need for work to directly evaluate the impact and potential mechanisms of action of collagen ingestion and more prolonged collagen supplementation on bone collagen synthesis in vivo in humans.

Few studies have evaluated the long-term impact of collagen supplementation on skeletal muscle mass and strength with and without exercise training. Recently, Mertz et al[135] demonstrated no impact of 20 g of collagen plus 10 g of carbohydrate supplementation vs 30 g of carbohydrate supplementation over 1 year on muscle mass or strength in healthy older men and women.

However, dietary-protein supplementation has been shown to augment the gains in skeletal muscle mass and strength after prolonged resistance-type exercise training.[5,136] In line with these findings, 2 long-term, resistance-type exercise training studies have reported that collagen peptide supplementation further augments training-induced skeletal muscle mass and strength gains. Zdzieblik et al[137] showed that daily 15-g collagen peptide supplementation resulted in an average increase of 4.2 kg of fat-free mass vs 2.9 kg in the placebo-supplemented group after resistance-type exercise training in men with sarcopenia. It has been recently highlighted[138] that the increase in fat-free mass observed in the collagen-supplemented group was exceptionally high (2.7-fold) in comparison with the increase in fat-free mass reported in recent meta-analyses examining the impact of protein supplementation on gains in fat-free mass after prolonged resistance exercise training.[5,136] However, the anabolic impact of collagen peptide supplementation was also more recently demonstrated by Oertzen-Hagemann et al,[139] who showed that 15 g of collagen peptide supplementation resulted in an average increase of 2.6 kg fat-free mass vs 0.7 kg in the placebo-supplemented group after resistance-type exercise training in young, healthy men. Though the reported gains in muscle mass and strength do not seem realistic, these findings underline the importance of performing additional studies with a well-controlled dietary intake approach to confirm the potential impact of collagen peptide ingestion on training-induced gains in muscle mass and strength.

Finally, Oikawa et al[140] recently demonstrated that collagen peptide supplementation (30 g, twice daily, ∼45% of total protein intake, 1.6 g protein/kg body mass/d) did not attenuate the loss of lower limb fat-free mass during energy intake restriction (−500 kcal/d) and a reduction in physical activity (< 750 steps/d) in older men and women. These findings seem to add to the general understanding that the anabolic potential of protein sources with low essential amino acid content (ie, collagen) are minimal.

Proposed mechanisms of action of collagen-derived protein supplementation

Glycine and proline as precursors for collagen synthesis. Provision of specific amino acids and peptides represent the 2 most likely mechanisms by which collagen-derived protein ingestion may upregulate tissue collagen synthesis and/or improve connective tissue function. As mentioned, the ingestion of relatively large doses of essential amino acids, casein, or whey do not seem to stimulate intramuscular connective tissue protein synthesis rates in vivo in humans.[11,14,25,28] Connective tissue proteins contain high levels of proline (12%) and glycine (25%) (Table 2) relative to other proteins within skeletal muscle.[32] Presumably, collagen protein synthesis rates will remain low or submaximal in the absence of sufficient glycine and proline availability. Early work has demonstrated that a reduction in glycine availability, as in the case of malnourished children,[141] may limit the synthesis of key proteins and peptides, such as glutathione.[142] More recently, Meléndez-Hevia et al[143] estimated that glycine availability from endogenous sources (ie, synthesized from serine and protein breakdown) and provided by a regular diet falls short of the estimated metabolic requirements, including glycine required to support tissue collagen synthesis. It may be suggested, therefore, that the amount of glycine (and proline) provided by a regular diet is insufficient for facilitating increased rates of tissue collagen synthesis. Therefore, the ingestion of protein sources rich in proline and glycine (Table 3) may be more suitable than high-quality protein sources, such as casein or whey protein (providing only ∼6% proline and ∼2% glycine) (Table 1) for supplying the specific amino acid precursors required to support de novo connective tissue protein synthesis.[29] In support of this, exposure to growth media containing proline and ascorbic acid has been shown to increase collagen content and improve the mechanical properties of engineered ligaments in an in vitro setting.[91] In rats, Vieira et al[92] showed that a diet rich in glycine further improved hydroxyproline content and maximal tolerable load in an Achilles tendonitis model, suggesting enhanced tendon recovery. Aside from its potential role as an amino acid precursor for collagen synthesis, glycine administration restored the activity of key translational signaling proteins (ie, mTORC1 pathway) in response to leucine administration in a rodent inflammation model.[144,145] These findings may indicate the potential for glycine (or collagen-derived protein) supplementation to increase the muscle protein synthetic response to food intake in clinically compromised individuals with low-grade inflammation.[146]

Collagen-derived protein uniquely contains hydroxyproline (13.5%). The role of hydroxyproline provision in facilitating connective tissue protein synthesis remains unclear. No codon exists for hydroxyproline, and its content in collagen is the result of a posttranslational modification of proline. Therefore, a postprandial increase in plasma free hydroxyproline due to collagen-derived protein ingestion cannot be used as precursor for incorporation into newly synthesized procollagen.[147] Whether increased hydroxyproline availability possesses stimulatory properties for collagen synthesis remains unknown. Ultimately, the impact of greater glycine, proline, and hydroxyproline provision through

dietary collagen-derived protein supplementation on in vivo connective tissue reconditioning in humans remains to be addressed.

Collagen peptides to facilitate connective tissue remodeling. It has been proposed that ingestion of collagen-derived peptides may directly enhance connective tissue remodeling. As described previously, evidence has been presented in some studies to suggest that collagen-derived di- and tripeptides (eg, Pro-Hyp, Hyp-Gly, Pro-Hyp-Gly) are absorbed in their intact form, albeit in low concentrations, into the circulation. However, currently, no human studies have evaluated the impact of the ingestion of these collagen-derived peptides on connective tissue synthesis or other in vivo remodeling processes (ie, cross-linking, breakdown), to our knowledge. Collagen-derived peptide administration has been shown to stimulate collagen synthesis and cross-linking activity and decrease breakdown using in vivo rodent and in vitro models. Oesser et al[104] showed, with the use of specially produced ^{14}C-labelled collagen peptides, that amino acids derived from orally administered peptides are incorporated in skin, liver, spleen, and skeletal muscle tissue in mice. However, the amino acids derived from administered peptides were most effectively delivered to cartilage tissue, as evidenced by nearly 2-fold greater specific radioactivity in comparison with oral administration of free ^{14}C-proline.[104] On the basis of these findings, it was suggested that amino acids ingested in peptide form are more effectively incorporated into cartilage than are their free amino acid counterparts. Most studies evaluating the impact of collagen peptides on connective tissue remodeling responses have been performed using skin models. For instance, Zague et al[148] showed that 4 weeks of collagen hydrolysate supplementation increases collagen types I and IV concentrations and decreased MMP-2 activity in rat skin. More recently, Zague et al[149] showed that exposure to collagen peptides increases collagen I synthesis and inhibits MMP-1 and MMP-2 activity in human skin collected during elective surgery. Edgar et al[106] showed that collagen peptide exposure to human-derived skin cells increases elastin synthesis and decreases synthesis of MMP-1 and MMP-3 along with the elastin degradation product desmosine. The impact of collagen peptide exposure to enhance remodeling may extend to other tissues. For instance, Yamada et al[150] showed that fish-derived collagen peptide exposure increases mixed collagen content along with COL1A2 mRNA expression in osteoblasts. The mRNA expression of several lysyl oxidase isoforms were also evaluated, revealing upregulation of some (namely, LOX-2, -3, and -4), but not the predominant isoforms (LOX, LOX-1).

Ohara et al.[151] evaluated 9 different collagen-derived peptides in vitro and showed that only exposure to Pro-Hyp stimulated proliferation of human dermal fibroblasts. This finding aligns with those of Shigemura et al,[152] who showed that exposure to Pro-Hyp stimulated the growth of mouse skin fibroblasts when embedded in a collagen gel and exposed to various growth factors. However, it is important to note that no stimulatory effect was demonstrated after exposure of 50 μmol/L Pro-Hyp, which has been reported in humans as the peak plasma concentration after the ingestion of as much as 26 g of collagen peptides derived from fish scales.[122] Any stimulatory effect of collagen-derived peptides on fibroblasts may be restricted to only Pro-Hyp; 2 studies reported no impact of mixed collagen peptide exposure on human dermal fibroblast[149] or mouse osteoblast proliferation.[150]

Although these studies suggest that collagen peptide provision may stimulate collagen synthesis and limit breakdown activity, the findings are unlikely to carry over to the human in vivo setting for various reasons. First, the in vitro setting tests fibroblasts directly on the culture plate or within a collagen gel. In the in vivo setting, fibroblasts are embedded in the ECM. Within the matrix, fibroblasts are situated optimally to sense and respond to metabolic and mechanical stimuli, such as an elevation in growth hormone concentrations or amino acids released from collagen breakdown. In the musculoskeletal tissues, fibroblasts are embedded in a longitudinal orientation to sense mechanical loading during physical activity. The in vitro experiments are often conducted in the absence of mechanical loading, under the administration of supraphysiological concentrations of nutrients and growth factors known to upregulate fibroblast proliferation, activity, and collagen synthesis. The importance of the in vivo environment is exemplified by the work of Shigemura et al,[152] who clearly demonstrated that peptide administration increased collagen synthesis only when fibroblasts were incubated for at least 96 hours in optimized growth medium (ie, 5%–10% fetal bovine serum, rich in platelet-derived growth factor and FGF-1). As such, the absence of mechanical stimuli known to enhance fibroblast proliferation, activity, and/or collagen synthesis and breakdown in the in vitro model likely affected the results. Second, the concentrations of collagen-derived peptides provided to cells in nearly all studies are $>$ 4-fold higher than what has been reported to be released in the plasma circulation after the ingestion of $\sim$25 g collagen peptides in humans.[152] Shigemura et al[152] did not detect an effect of Hyp-Pro on fibroblast proliferation when Hyp-Pro was administered in an amount that more closely reflected peak plasma concentrations. Last, incubation times of peptides in these in vitro

studies generally ranged between 24 hours and 6 days.[106,149,151,152] In humans, the postprandial period of protein digestion and absorption is far shorter, with several studies demonstrating that peptide concentrations may only be increased from 4 to 6 hours after ingestion.[126,153,154] Therefore, it is expected that any stimulatory effect of peptide ingestion is minor and/or short-lived in comparison with the responses reported in the in vitro setting. Of course, no conclusions on the proposed impact of collagen peptide ingestion on connective tissue remodeling can be reached until evidence is gathered in the in vivo human setting.

CONCLUSIONS

Musculoskeletal connective tissue networks are in a constant state of remodeling. Exercise increases intramuscular connective tissue protein synthesis rates, demonstrating that connective tissue protein possesses a high level of plasticity. Despite the anabolic properties of dietary protein on muscle tissue remodeling, no study has demonstrated the impact of dietary protein ingestion to increase connective tissue synthesis rates. However, these studies have only evaluated the connective tissue protein synthetic response to essential amino acid and dairy protein ingestion. It has been suggested that the ingestion of collagen-derived protein sources, such as collagen peptides or gelatin, may be more suitable for stimulating connective tissue protein synthesis. Collagen-derived proteins contain ample amounts of glycine and proline, which may facilitate an increase in connective tissue protein synthesis rates. Some have speculated that collagen-derived protein sources also contain peptides with stimulatory or inhibitory properties. Although some in vitro evidence exists to support this hypothesis, there are no data, to our knowledge, to support the claim that the ingestion of collagen-derived peptides may stimulate connective tissue protein synthesis rates. Research is warranted to establish the proposed benefits of collagen-derived protein ingestion as a means to support connective tissue remodeling within musculoskeletal tissues. Such evidence is necessary for the potential development of nutritional intervention strategies to increase muscle strength and function in a variety of populations, including athletes and older individuals.

Acknowledgments

Author contributions. A.M.H. and L.J.C.vL conceived the idea of the review. A.M.H. compiled the articles and extracted the data, wrote the manuscript, and generated tables and figures. L.J.C.vL critically revised the manuscript. Both authors read and approved the final manuscript. Figures were created with Biorender.com.

Funding. A.M.H and L.J.C.vL have received funding from PB Leiner, part of Tessenderlo Group, to study the role of dietary collagen on skeletal muscle health. L.J.C.vL and his laboratory have received research grants, consulting fees, speaking honoraria, or a combination of these for work on postprandial protein muscle metabolism; a full overview is provided at https://www.maastrichtuniversity.nl/l.vanloon.

Funders did not play a role in the conception, design, performance, and approval of the presented work.

Declaration of interest. The authors have no relevant interests to declare.

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