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# Remodelling of Skeletal Muscle Extracellular Matrix: Effect of Unloading and Reloading

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Additional information is available at the end of the chapter

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## Abstract

This chapter summarizes studies that examine remodelling of extracellular matrix (ECM) and role of regulatory factors of ECM during unloading and reloading. Hypokinesia has a catabolic effect on both the contractile apparatus and ECM of the skeletal muscle, causing the formation of muscle atrophy, the decrease of the synthesis of contractile proteins and disturbance of the collagen metabolism. The metabolism of fibrillar and non-fibrillar collagens in ECM plays a crucial role in exercise and sport, influencing the strength development through transmission of contractile force in skeletal muscle. The impairment of motor activity and muscle strength is accompanied by the muscle atrophy. The muscle atrophy caused by inactivity and recovery from atrophy demonstrates the plasticity of muscle. Muscle mass and volume increase in a relatively short time, but the recovery of strength takes much longer and is related with the regeneration of the muscle structures. The recovery period of the contractile apparatus and ECM structures is different in slow- and fast-twitch skeletal muscle.

Although the muscle tissue's response to inactivity is more pronounced than the response of ECM, important changes occur in the connective tissue structures during unloading, causing the impairment of the functional characteristics of the skeletal muscle.

**Keywords:** ECM, regulatory factors of ECM, unloading, reloading, functional characteristics of skeletal muscle

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## 1. Introduction

The intramuscular connective tissue accounts for 1–10% of skeletal muscle and has multiple functions [1,2]. It provides a basic mechanical support for vessels and nerves. The connective tissue ensures a passive elastic response of the muscle [1,3,4].

It is important to accept that both the tendon and the intramuscular connective tissue interact closely with the contractile elements of the skeletal muscle to transmit force [5–7]. The force transmission from the muscle fibres is not only transformed to the tendon and the subsequent bone via the myotendinous junctions, but also via the lateral transmission between neighbouring fibres and fascicles within the muscle [1,6]. The tension developed in one part of the muscle can be transmitted via shear links to other parts of the muscle. The perimysium is especially capable of transmitting tensile force [3,7].

The extracellular matrix (ECM) is formed by complex molecular networks, which determine the architecture of a tissue and regulate various biological processes [6,8]. The skeletal muscle ECM is organized in three levels: the epimysium surrounds the entire skeletal muscle, the perimysium surrounds muscle bundles consisting of a variable number of muscle cells, and the endomysium outlines the individual muscle fibres [2,7]. The ECM consists of various substances, of which collagen fibrils and proteoglycans are the most widespread [3,9]. The most abundant protein of the extracellular matrix is collagen, accounting for 20–25% of all protein in the whole body and forming more than 90% of the organic mass of bone [2,6]. At present, 26 different collagen types have been identified [2,10]. Although the impact is not well established, various isoforms of collagen exist, and they have varying strength and functional characteristics [6]. Muscular flexibility is partly provided by collagen. For this purpose the organization of fibrils and fibres is critical, because individual collagen molecules, fibrils, and fibres are intrinsically inextensible [2,11]. The extensibility of collagen results from the straightening of curved fibrils and fibres [2]. In addition to the proteoglycans, the hydrophilic ECM includes several other proteins such as noncollagen glycoproteins [9].

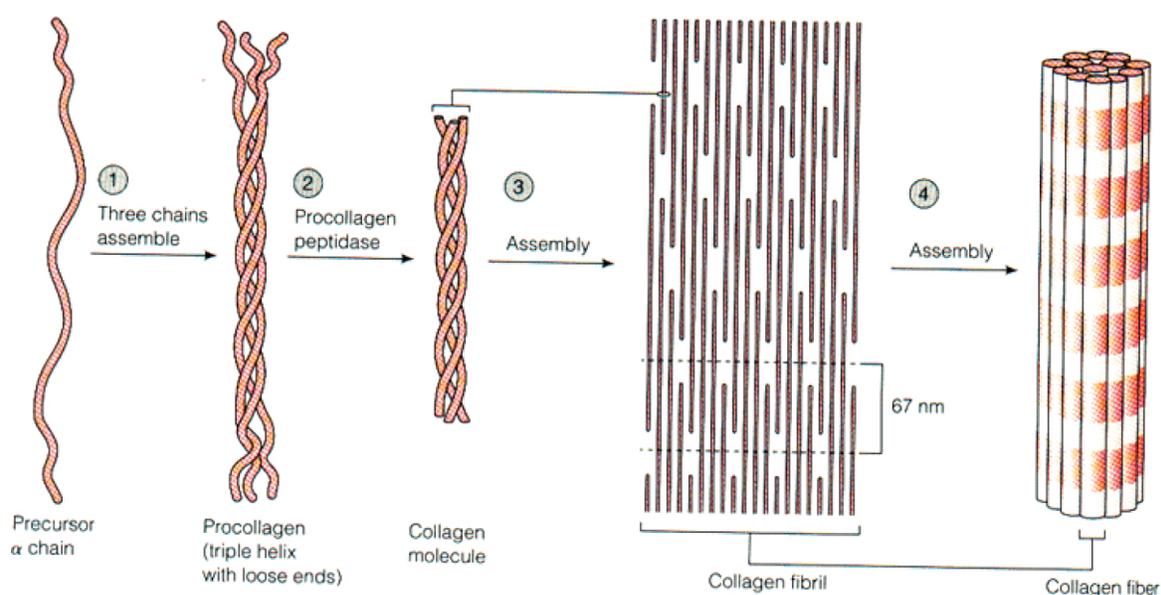
## 2. Functions and composition of ECM in skeletal muscle

### 2.1. Fibril forming collagen types in skeletal muscle

Type I and III collagen are the most abundant fibril forming collagens in the skeletal muscle. Type I collagen dominates in the intramuscular collagen content – reported from 30% up to 90% of total collagen [10,12]. The epimysium consists mainly of type I collagen with minor amounts of type III collagen [9–11]. Equal amounts of both collagen types are found in the perimysium. In the endomysium, type III collagen is the predominant form and only small amounts of type I collagen are found [2,11]. The smaller average diameter of oxidative muscle fibres should result in a higher endomysial connective tissue content in slow-twitch muscles, as the surface area/volume ratio of each fibre is greater than the average glycolytic fibre in fast-twitch muscle [3]. Slow muscles contain more type I collagen than type III collagen, the proportion of type III collagen is greater in fast muscles [13].

Type I collagen is the major stress-tolerant fibrillar collagen in the muscle. It has a high tensile strength and limited elasticity and is thus well-suited for force transmission [1,2]. Type III collagen, the other main fibrillar collagen, has a structure and arrangement similar to that of type I collagen, but it forms thinner and more elastic fibres. The fibres of type III collagen can also form copolymers with those of type I collagen [7]. Collagens I and III are fibril forming

and serve as a supportive structure in the muscle tissue. They attach myocytes and muscle bundles to each other [1,2,11]. Also nerves and capillaries are surrounded and attached to muscle by collagen [11]. Type V collagen is also fibril forming and can be found in the endo- and perimysium in smaller amounts than the collagen types I and III [2,10]. Collagens III and V are known to copolymerize with type I collagen and they may have a role in collagen fibre diameter regulation [6,10]. Type V collagen is considered to form the core of the fibrils, and collagens I and III copolymerize around this core [2,3]. Type II and XI collagens are also fibril forming and have been detected in the skeletal muscle only at mRNA level [3,10]. Type V and XI collagens form heterotypic molecules and can be considered as a single kind of collagen [2, 10]. Fibril associated collagens with interrupted helix (FACIT) types XII and XIV are located only in the perimysium [2,14]. These FACIT collagens associate with the surface of interstitial collagen fibrils and possibly act as molecular bridges among or between fibrils and other components of the ECM [2,9]. Although mRNAs of the other members of FACIT subfamily (IX, XVI, XIX, XXI) are detected in the skeletal muscle, the respective proteins have not been found [14,15]. The formation process of fibrillar collagen is depicted on **Figure 1**.



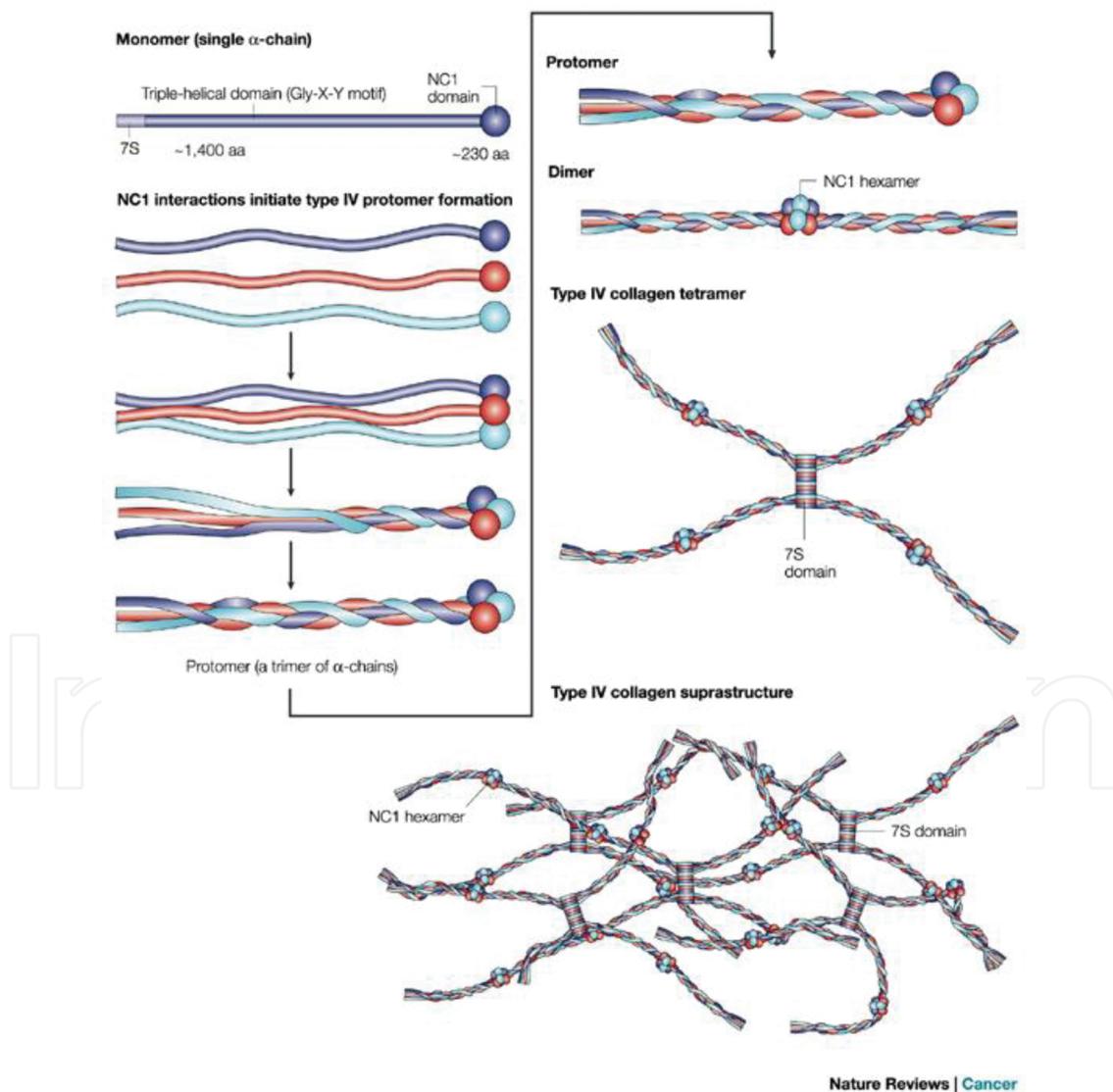
**Figure 1.** The formation process of fibrillar collagen.

## 2.2. Nonfibrillar collagen types of skeletal muscle.

Nonfibrillar collagens of the skeletal muscle are mainly located in the basement membranes. The basement membrane (BM) is a highly specialized sheet of the connective tissue surrounding individual muscle fibres, blood vessels, Schwann's cells and the spindle capsule cells. The components of the BM are the regulators of many biological activities such as cell growth, differentiation and migration which influence tissue development and repair [2,6,16]. Integrins attach muscle cells to ECM and serve as the force-transmitters between ECM and the con-

tracting components inside the muscle cells. They connect laminin to the cell membrane to form the inner layer of basement membrane [3,17].

Type IV collagen is a major component in the basement membrane and therefore plays a critical role in the cellular arrangement in the muscle tissue. It is an integral component of basement membrane and forms a covalently stabilized polymer network around the muscle fibres [2,10]. Type IV collagen molecules form a mesh-like structure outside the laminin layer and give stability to the BM [18]. Laminin and type IV collagen are connected to each other by nidogen-1 in the muscular basement membranes [2,19]. As a part of the flexible basement membrane, type IV collagen network is interconnected with other extracellular matrix compounds and sarcolemmal proteins, being consequently exposed to stretching effects during muscle contraction [20,21]. The formation process of type IV collagen is demonstrated in **Figure 2**.



**Figure 2.** The formation process of type IV collagen.

Type VI collagen interacts with type IV and type I collagens [2,10], providing a link between the basement membranes and the surrounding matrix. Collagens XV and XVII belong to the multiplexin subfamily of nonfibrillar collagens [16] and are located in the basement membrane zone [2,10,16]. Collagens XV and XVIII may have a role in stabilizing the muscle cells [2,10]. Type XIII collagen is the transmembrane protein which is capable of binding certain basement membrane proteins [2,22]. It probably provides a link between the muscle cell and its basement membrane [10]. Type XIII collagen is concentrated in the myotendinous junctions [10,22].

### 3. Collagen synthesis in skeletal muscle

The synthesis of collagen is similar to other proteins, consisting of genetic transcription with messenger ribonucleic acid (mRNA) and ribosomal translation of the mRNA to prepro  $\alpha$ -chains. In the skeletal muscle, collagens are expressed principally by fibroblasts, and their biosynthesis is characterized by the presence of an extensive number of co- and posttranslational modifications of the polypeptide chains [10,23]. Gross fractional synthesis rate for collagen is about 5% a day in the skeletal muscles of young adult rats (), whereas the fractional synthesis rate for total protein is about 11–15% /day [24].

Collagen is a protein with three polypeptide chains where each chain contains at least one stretch of the repeating amino acid sequence  $(\text{Gly-X-Y})_n$  and X and Y can be any amino acid (often proline and hydroxyproline, respectively). Both fibrillar and non-fibrillar collagens consist of three long polypeptide chains, which may or may not be identical and combine together via their  $(\text{Gly-X-Y})_n$  sequences to form a collagen triple helix. The molecular organization of different collagen types differs so that type I collagen is a heterotrimer of two identical  $\alpha 1(\text{I})$  chains and one  $\alpha 2(\text{I})$  chain, whereas type III collagen is a homotrimer with  $\alpha 1(\text{III})$  chains [2,6]. The repeating unique amino acid sequence Gly-X-Y, where the glycine is in every third position, has no interruptions in the fibril-forming collagen types, whereas a considerable number of interruptions occurs in the nonfibrillar collagens [2,10]. The Gly-X-Y repeat unit gives requirements for coiling the three  $\alpha$ -chains tightly around one another. Proline and 4-hydroxyproline residues appear frequently at the X- and Y positions, respectively, and promote the stability of the triple-helix and the structure of collagen as a whole. The structure of type IV collagen genes is distinctly different from those of fibril forming collagens. The most common form of type IV collagen consists of two  $\alpha 1(\text{IV})$  chains and one  $\alpha 2(\text{IV})$  chain, although the combinations of  $\alpha 3(\text{IV})$  and  $\alpha 4(\text{IV})$  as well as  $\alpha 5(\text{IV})$  and  $\alpha 6(\text{IV})$  are found in some basement membranes [2,10].

#### 3.1. Modifications of the polypeptide chains

An exception to the synthesis of other proteins is that collagen synthesis is characterized by an extensive number of co- and posttranslational modifications of the polypeptide chains. The intracellular modifications of polypeptide chains involve hydroxylation and glycosylation reactions to form the procollagen. Hydroxylation of proline, the reaction catalyzed by prolyl 4-hydroxylase (P-4-H), influences the stability of the triple-helical structure of collagen [20]. The triple-helix formation of the pro- $\alpha$ -chains prevents any further hydroxylation. Intracellu-

lar events of collagen synthesis include also 3-hydroxylation of proline residues, hydroxylation of lysine residues and glycosylation of certain hydroxylysine residues of propeptides. The assay of prolyl-4-hydroxylase activity has been commonly used to estimate the changes in the rate of collagen synthesis [20]. Fibrillar collagens are secreted as soluble procollagens, which are converted to collagen by the cleavage of C- and N terminal propeptides by procollagen N- and C-proteinases. Extracellular modifications in the collagen synthesis involve an ordered self-assembly for the formation of collagen fibrils and the crosslink formation to make the fibrils stable. The stabilization of the fibrils is provided by covalent cross-links generated by the conversion of some of the lysine and hydroxylysine residues to aldehyde derivatives by lysyl oxidase [2,25]. Lysyl oxidase (LO) is a key enzyme in the extracellular modification of collagen [25]. LO, an amine oxidase expressed and secreted by fibrogenic cells, plays a critical role in the formation and repair of the ECM by oxidizing lysine residues in elastin and collagen, thereby imitating the formation of covalent crosslinkages which stabilize these fibrous proteins [25]. Type IV collagen molecules form their network with different processes. A tight meshwork is formed by irregularly branching lateral associations of the triple helical regions [2,10]. The formation steps of collagen is demonstrated in **Table 1**.

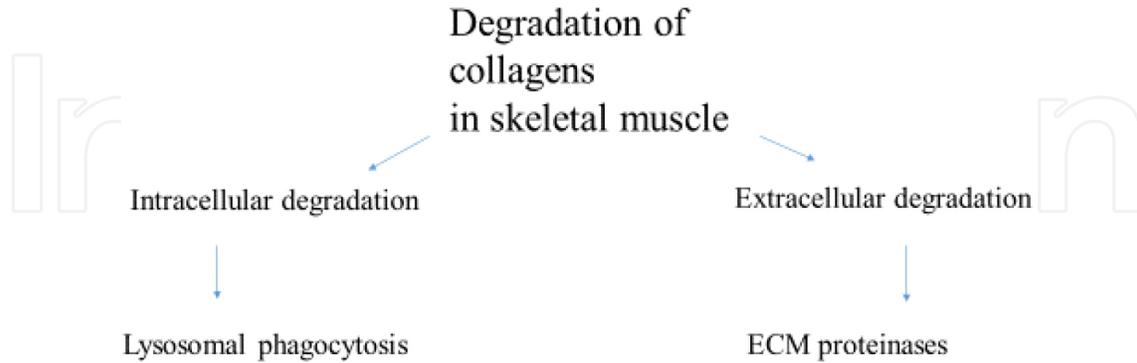
NUCLEUS	Collagen gene → RNA processing → mRNA
CYTOPLASM Rough endoplasmic reticulum	Pre-procollagen chain → cleavage of signal peptide
Golgi apparatus	Procollagen α chain → hydroxylation – glycosylation – association of the C-terminal propeptides - disulfide bond formation → procollagen molecule
EXTRACELLULAR MATRIX	Cleavage of propeptides → collagen fibril self assembly → crosslinking

**Table 1.** The formation steps of collagen.

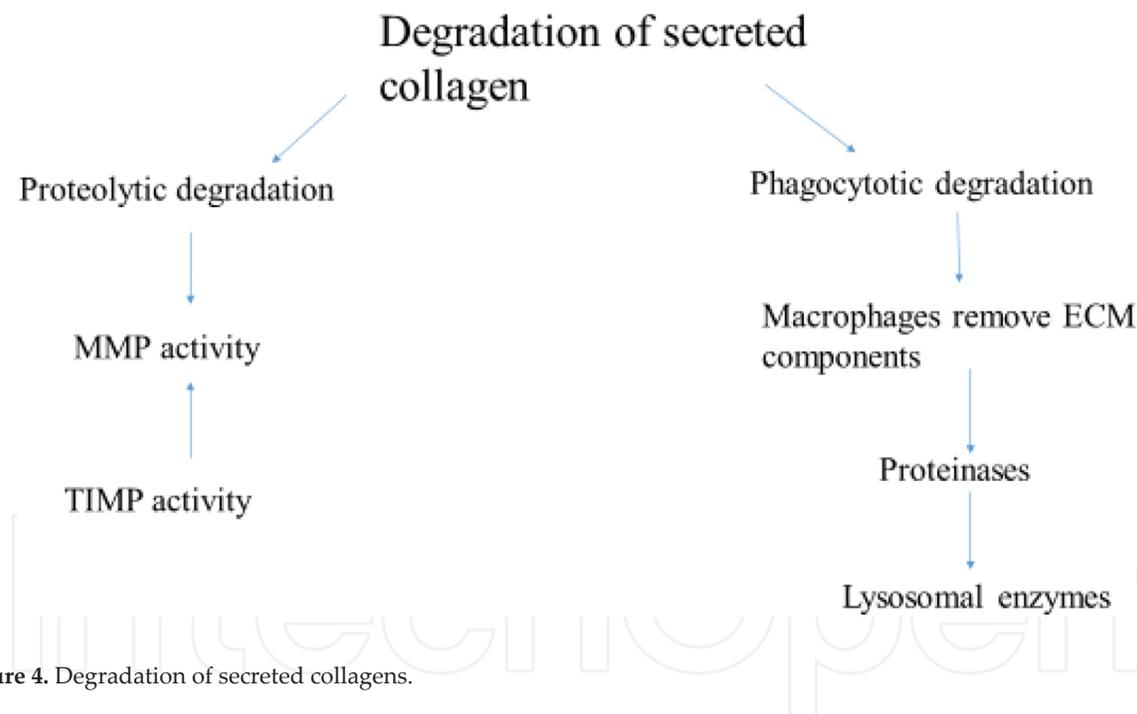
#### 4. Degradation of collagens in skeletal muscle

Degradation of collagen represents the obligatory step of a turnover and the remodelling of the connective tissue and during the mechanical loading of fibroblasts and extracellular matrix structures. Both intracellular and extracellular degrading pathways are present, using either lysosomal phagocytosis or ECM proteinases, respectively [26,27]. Collagens can be degraded prior to or after their secretion from the cell. Secreted collagen is degraded mainly by two different routes: proteolytic and phagocytotic. Proteolytic degradation occurs mainly through matrix metalloproteinase (MMP) activity. Macrophages remove ECM components, although also fibroblasts are able to the phagocytosis and degradation of collagen fibrils [27]. Degradation is continued by specific proteinases and the collagen fragments are phagocytosed by cells and processed by lysosomal enzymes [28]. About 26% of newly synthesized collagen is

degraded per day in young adult rats [24]. The most recently synthesized collagen seems to be more susceptible to degradation than mature collagen [10,24]. The main steps of collagen degradation are depicted in **Figure 3** and **4**.



**Figure 3.** Degradation of collagens in skeletal muscle.



**Figure 4.** Degradation of secreted collagens.

#### 4.1. Role of matrix metalloproteinases

Collagen degradation is initiated extracellularly by MMPs or matrix metalloproteinases, which are presented in tissues mostly as latent proMMPs [29,30]. MMPs are a family of zinc-dependent proteolytic enzymes that function mainly in the ECM [30,31]. The activation of specific matrix metalloproteinases has been implicated in degradative and atrophic changes in the ECM after muscle injury or in various myopathic conditions. These matrix metalloproteinases may cause structural and physiological alterations to the basal lamina and sarcolemma

of myofibres, leading to uncontrolled influx and efflux of ions and subsequent myopathy [31, 32]. Because of their ability to degrade ECM components, MMPs are considered to be important components in many biological and pathological processes [30–32]. They have regulatory roles in muscle growth and development and are also important in repair processes after traumatic injury or disuse myopathy [30,31]. MMPs are mainly produced from endotendon fibroblasts and intramuscular matrix fibroblasts [30,31], although some level of expression has been found to occur also in satellite cells [33]. MMPs are secreted or released in latent form and become activated in pericellular environments [23,34]. The activities of MMPs are also under the control of enzyme tissue inhibitors of matrix metalloproteinases (TIMPs). Disturbances in the ratio of specific MMPs and their inhibitors may be manifested by physiological dysfunction, resulting in clinical disorders [31,35].

Up until now, 24 different vertebrate MMPs have been identified, of which 23 have been found in humans. MMPs are usually divided according to their main substrate into collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others, although many of them have wide and overlapping substrate specificity [36].

MMP-1, MMP-8, MMP-13 and MMP-18 are collagenases, which have the ability to cleave the native helical structure of collagens I, II and III. Cleavage products are then susceptible to the action of other MMPs [36,37].

Gelatinases MMP-2 and MMP-9 degrade denatured collagen, gelatin, native type IV, V and VII collagens as well as other ECM components [36]. One of the most important MMPs associated with the function and dysfunction of the skeletal muscle appears to be MMP-2, also known as gelatinase A, or 72-kDa type IV collagenase. MMP-2, by regulating the integrity and composition of the ECM in skeletal muscle, plays essential role in myofibre proliferation and differentiation, the fibre healing after injury, and maintenance of the surrounding connective tissue [38]. MMP-2 also digests fibrillar type I and II collagens. MMP-2 and –9 are known to be overexpressed and present in higher amounts in patients with inflammatory myopathies, which may increase ECM degradation and thus facilitate lymphocyte adhesion [32,38,39].

MMP-3 and MMP-10, or stromelysin-1 and –2, both digest ECM components and activate proMMP-1. The third stromelysin, MMP-11, differs from other stromelysins by its sequence and substrate specificity [36].

Matrilysins- MMP-7 and MMP-26 are the smallest MMPs. MMP-7 can also process cell surface molecules [36].

Six membrane-type MMPs (MT-MMPs) have been characterized. Except the MT4-MMP, they all are all capable to activate proMMP-2 [36,40]. For their pericellular fibrinolytic activity, MT-MMPs have an important role in angiogenesis [36,40].

Six MMPs – MMP-12, MMP-19, MMP-20, MMP-22, MMP-23, MMP-28 are currently classified into the group of “other MMPs” [34,36].

#### **4.2. Inhibition of matrix metalloproteinases by tissue inhibitors of metalloproteinases**

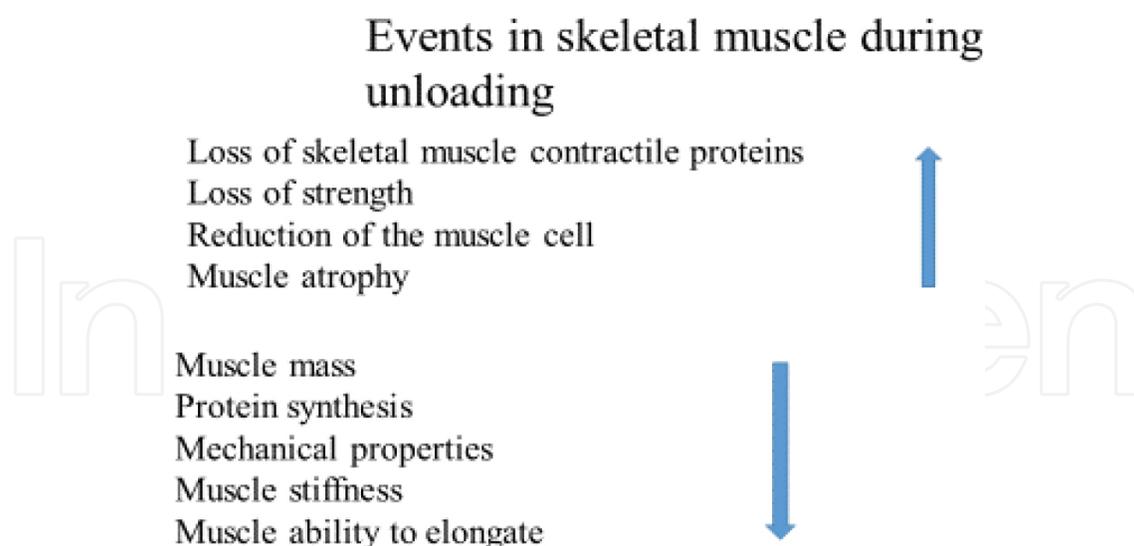
MMPs and tissue inhibitors of metalloproteinases (TIMPs) have an important role in the adaptive changes in the muscle in response to local developmental, physiological, surgical, and pathological conditions [31,39]. TIMPs are the major cellular inhibitors of the MMP sub-family, exhibiting varying efficacy against different members, as well as different tissue expression patterns and modes of regulation [36,41]. Four mammalian TIMPs have been characterized and considered to regulate MMP activity during tissue remodelling [41,42]. All four TIMPs (TIMP-1, -2, -3 and -4) can inhibit all MMPs, except TIMP-1, which is a poor inhibitor of MMP-19 and most of the MT-MMPs [41,42]. Although TIMP-2 inhibits MMP-2 in high concentrations, it has an important role in activating proMMP-2 in a complex with MT1-MMP, which demonstrates an integrated response of MMPs and TIMPs [43]. In skeletal muscle, TIMP-1, TIMP-2 and TIMP-3 are expressed [33,39]. TIMP-4 appears to be cardiac-specific and has not been detected in the skeletal muscle [37].

The increased MMP activity and thus the enhanced degradation of collagen often parallels the stimulated activation of collagen synthesis. TIMPs are often activated together with MMPs in response to physical activity, indicating a simultaneous stimulation and the inhibition of degradation [44,45]. MMP activity precedes TIMP activity and thus TIMP serves as the regulator of degradation termination to ensure a limited amount of degradation [39,43].

In addition to MMP-binding activities, TIMPs have many important biological functions. TIMPs can promote or inhibit cell growth, depending on the type of the cell and the inductor [36,41].

### **5. Effect of unloading on the skeletal muscle**

The inactivity of the skeletal muscle leads to the loss of muscle contractile proteins and strength [46,47]. The weakening of the muscle is accompanied by the loss of the muscle mass and the reduction of the size of the muscle cell [46,48]. The decrease in the protein synthesis and the increase in protein degradation appear both in the contractile apparatus and in the ECM [8,46]. The skeletal muscle atrophy attributable to the muscular inactivity has significant adverse functional consequences, nevertheless the tight connections between the contractile machinery and the ECM are still unknown [27]. Changes in the intramuscular collagen protein fraction have been shown to significantly impact mechanical properties of skeletal muscle in non-loading conditions [27,49]. Events in skeletal muscle during unloading are shown in **Figure 5**.



**Figure 5.** Events in skeletal muscle during unloading.

### 5.1. Effect of unloading on the extracellular matrix

The ECM of connective tissues enables links to other tissues and plays a key role in force transmission and tissue structure maintenance in tendons, ligaments, bone and muscle [18]. ECM turnover is influenced by physical activity [50,51]. Immobilization causes a marked relative increase in the endo- and perimysial connective tissue, which results in changes of the mechanical properties of skeletal muscle [11].

Fibrillar type I and III collagens are most abundant in the skeletal muscle epi- and perimysium. Non-fibrillar type IV collagen is present only in basement membranes and has a critical role in the cellular arrangement of muscle tissue [2,11]. There are differences in the collagen metabolism and the content between muscles. Slow-twitch muscles contain 40–50% more collagen than fast-twitch muscles [13].

A reduced muscular activity decreases the collagen synthesis rate in the skeletal muscle, the immobilization down-regulates the collagen synthesis at the pretranslational level, mainly among I and III collagens [18,20]. Unloading also induces a shift in the relative proportion of collagen isoform type I to III [13]. Decrease of collagen I mRNA level in slow-twitch Soleus (Sol) and fast-twitch gastrocnemius (GM) muscle during the three-week hindlimb suspension shows that the fibrillar type I collagen is more sensitive to unloading and the effect is much more long-lasting than that of fibrillar type III collagen. This finding shows that hindlimb unloading induces reduction of collagen type I [52].

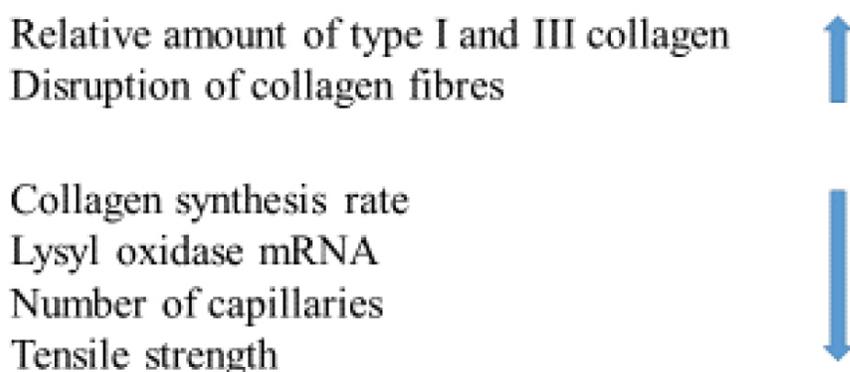
Lysyl oxidase which plays an important role in the formation and regeneration of ECM by oxidizing lysine residues in elastin and collagen, initiates the formation of covalent cross-linkages which stabilize fibrous proteins [25]. From this standpoint it is understandable that a significant decrease in LO mRNA level was registered only in Sol muscle [25].

Matrix metalloproteinases are providing degradation of ECM compounds [31,36]. MMP-2 level did not change significantly during three weeks of hindlimb suspension. TIMPs are proteins which inhibit ECM degradation [35,53,54]. The mRNA level of TIMP-1 decreased in slow-twitch muscle after one-week hindlimb suspension. As both intracellular (lysosomal phagocytosis) and extracellular degrading pathways (ECM proteinases) are present in the degradation of the skeletal muscle during the unloading, it is complicated to put all the role to the MMPs in this process [6].

The biggest changes in the specific mRNA level of type I, III and IV collagen were registered in Sol and GM muscle during the three weeks of unloading mRNA level of LO decreased also in Sol muscle [52]. Changes in TIMP-1 mRNA level during first week of hindlimb suspension were contradictory in Sol and GM muscle [52].

The metabolism of fibrillar and non-fibrillar collagens in ECM plays a crucial role both in decreased locomotory activity and in exercise and sport, influencing the strength development through transmission of contractile force in skeletal muscle. Events in ECM during unloading are shown in **Figure 6**.

## Events in extracellular matrix during unloading



**Figure 6.** Events in the ECM during unloading.

### 5.2. Effect of unloading on the synthesis of collagen

Several quantitative and qualitative changes in the intramuscular connective tissue contribute to the deteriorated function and biomechanical properties of the immobilized skeletal muscle [6,11,51]. Muscle and tendon collagen and the connective tissue network are known to respond to altered levels of physical activity [51,55]. In contrast to physical loading, immobilization of rat limb leads to a decrease in the activities of collagen synthesizing enzymes both in skeletal muscle and tendon [20,56].

Along with the increased amount of intramuscular connective tissue, the number of capillaries decreases dramatically [57,58]. Each capillary is surrounded by a dense layer of the connective

tissue fibres, isolating the capillary from the adjacent muscle fibre, which disturbs the blood supply of the muscle fibres and further increases the muscle fibre atrophy [57,58].

During immobilization, the normal three-dimensional orientation of the collagen fibres is disrupted. The normal orientation constitutes of the fibres running parallel to the muscle fibres on their surface, preventing muscle cells from over-elongation and –contraction. In addition, thin perpendicular fibres connect adjacent muscle fibres to each other [7,10,11]. As a result of decreased loading, the number of longitudinal fibres increases, the crimp angle of the collagen decreases and this diminishes the ability of the muscle to elongate [2,11] and because of that the skeletal muscle shows significantly decreased tensile strength [1,3]. In addition to changes in collagen abundance, alterations in the degree of collagen cross-linking would have a profound effect on the mechanical properties of skeletal muscle, causing a decrease in muscle stiffness [10,17].

In contrast to physical loading, immobilization leads to the decrease in the enzyme activities of collagen biosynthesis, which suggests that the biosynthesis of the collagen network decreases as a result of reduced muscular and tendinous activity [8,56]. The rate of the total collagen synthesis depends mostly on the overall protein balance of the tissue, but it seems to be positively affected by stretch in both muscle and tendon [55].

Collagen expression during immobilization has been shown to be at least partially down-regulated at the pretranslational level [55]. Although the relative amount of the connective tissue increases during immobilization, the gene expression of type I and III collagens decreases during the first three days of immobilization [20]. The content of type IV collagen was also reduced as a result of immobilization [23]. The activities of prolyl 4-hydroxylase (P 4-H) and galactosylhydroxylsyl glucosyltransferase (GGT) decrease from the first three days of immobilization up to at least three weeks, suggesting decreased collagen biosynthesis during that time [20,59]. The degradation of collagens has been found to be enhanced during immobilization, as the expression of both MMP-2 and MMP-9 increased after 30 days of immobilization [30]. The quantity of TIMP-1 was also increased after 30 days of immobilization [30].

The collagen concentration increases when expressed both as a function of muscle dry weight or muscle cross-sectional area, but this increase in muscle collagen is primarily due to the muscle atrophy induced by immobilization [13].

### **5.3. Effect of unloading on the contractile apparatus of skeletal muscle**

As the skeletal muscle is a highly plastic tissue, the conditions associated with the disuse are accompanied by adaptation. A period of time without weight bearing cause modifications of structure and the function of skeletal muscles, of which atrophy and a slow-to-fast transition are the most prominent [60,61]. Many animal models such as the kind limb suspension, immobilization in shortened and lengthened position, spaceflight and denervation show that the removal of a mechanical load produces atrophy and contractile alterations, more evident in the slow muscle soleus than in fast muscles as extensor digitorum longus [60,62,63]. Besides space flight and bed rest experiments, long periods of muscle disuse in relation to a disease or

traumatic injuries of the joints or of the bones are relatively common experience for human beings [62,63]. The inactivity causes only small increases in contractile speed and myofibrillar adenosine triphosphatase (ATPase) activity and slight elevations in the percentage of the fast type myosin heavy chain (MyHC) isoforms in fast-twitch muscles, as compared to slow-twitch muscle [64,65].

#### **5.4. Slow-to-fast transition in skeletal muscle during unloading**

The different response can be explained, considering the fact that skeletal muscles in different parts of the body are subjected to different patterns of recruitment and activity [66,67]. The anti-gravitational Sol muscle is recruited for prolonged periods at a moderate level of intensity, whereas the extensor digitorum longus muscle is less frequently recruited, performing short, high-force contractions [68,69]. It is commonly known that inactivity affects the functional and biochemical properties of antigravity muscles, causing a significant decrease in both contraction and relaxation times [66,70] and a significant increase in the maximal shortening velocity and myofibrillar ATPase activity [71]. The above-mentioned changes are considered to be a result of the gene expression, especially the genes involved in the fibre type transformation [71–73]. The co-ordinated changes in the gene expression are particularly apparent for myosin and consequently the disuse induces a slow-to-fast transition, as reflected by an increase in fast MyHC isoforms at the expense of slow MyHC in the Sol and a fast-to-faster MyHC shift in the GM muscle [74,75]. An increase in fast MyLC isoforms, an increased proportion of fast troponin subunits and hybrid fibres co-expressing fast and slow MyHC and MyLC appears during slow-to-fast transition in Sol muscle [64,71,76]. The fibre type transition results in a change in muscle metabolism, fuel use, and more fatigable muscle [77]. Several histochemical analyses have also suggested that the functional changes in immobilized muscles are due to an increase in fast-twitch IIA fibres [64]. In addition to above-mentioned facts, the increased sarcoplasmic reticulum calcium-ATPase activity and the preferential loss of thin filaments all contribute to faster contractile properties of the Sol muscle [78]. The increased shortening velocity may be an attempt to compensate for the loss of power generating capacity during unloading caused by weakening [79].

Muscle disuse is often accompanied by increased fatigability, which is caused by the reduced oxidative capacity of disused muscles [65,80,81]. Capillary loss and reduction in blood flow might contribute to the increased fatigability by an impaired supply of energy substrates and oxygen to the muscle [82].

#### **5.5. Formation of muscle atrophy during unloading**

While immobilization at shortened length induces atrophy, immobilization in lengthened position induces hypertrophy, which is largely attributable to addition of sarcomeres in the longitudinal direction [26,83]. Immobilization in the shortened position, e.g. hindlimb suspension, induces preferential transcription of fast MyHC isoforms, reminiscent of the slow-to-fast transition observed in other models of disuse [26,84]. The disuse atrophy is characterized by the loss of muscle mass and decrease of muscle diameter. In the case of muscle atrophy, there some noticeable changes in the muscle cell at the cellular level including sarcomere

dissolution and endothelial degradation, a marked reduction in the number of mitochondria, the accumulation of the connective tissue, the elimination of apoptotic myonuclei and a decrease in capillary density [85–87]. Selective susceptibility of fibre types to immobilization seems to exist, while the red muscle fibres show the greatest atrophy. The decreased synthesis of protein and increased protein degradation are characteristic features to muscle atrophy. At least half of the total muscle protein is myofibrillar protein, and this fraction is lost at a faster rate than other muscle proteins during atrophy [84]. Three major proteolytic systems to skeletal mass protein loss are the cytosolic calcium-dependent calpain system, the lysosomal proteases and the ATP-dependent ubiquitin-proteasome system, which work as partners during muscle proteolysis rather than one system being used exclusively during atrophy [88]. Recent advances in cellular biology show the oxidative stress to be an important regulator of pathways leading to muscle atrophy during periods of disuse, increasing the expression of the key components of the proteasome proteolytic system. This proteolytic system is a prominent contributor to protein breakdown in skeletal muscle during periods of inactivity [37,84,86].

### **5.6. Effect of unloading on the skeletal muscle MyHC composition**

Prolonged periods of time spent with a diminished or no-weight bearing have a deleterious effect on skeletal muscle with the decreased protein synthesis, the loss of muscle mass and alterations of biochemical parameters [74,77]. The main findings confirmed that the proportion of slow MyHC isoforms decreased and the proportion of MyHC fast isoforms increased in consequence of altered functional conditions [46,64,89].

Clinical observations show that the atrophy of the skeletal muscle occurs as a result of immobilization and is caused by the changed functional conditions in the muscular system. Skeletal muscle function depends on the intact proprioceptive activity, motor innervation, mechanical load, and joint mobility. If one of these factors is altered, the muscle will undergo adaptation. As an increased muscular activity leads to the enhancement of the structures involved in contraction, inactivity or disuse is followed by the reduction of the muscle mass [8,77].

Alterations of biochemical parameters and changes at the ultrastructural level of the contractile apparatus are considered to be characteristic of atrophied muscles. The effect of disuse on the skeletal muscle depends on the fibre type composition of the muscle. The degenerative changes in disused muscles at the ultrastructural level have been shown to be most severe in slow oxidative muscle fibres. It is suggested that the most vulnerable muscles were antigravity muscles crossing a single joint [90].

The properties of muscle contraction which depend on the MyHC isoform composition decrease in atrophied skeletal muscle [46,89,91]. Contractile activity can induce differential expression of myosin protein isoforms in skeletal muscle. MyHC composition has an important regulatory role in myosin ATPase activity and muscle fibre shortening velocity [92]. A prolonged activity causes alterations in the MyHC composition. A decrease in the mechanical load stimulates the conversion of slow myosin in muscles of mixed fibre type composition, whereas a decrease in the weight-bearing load results in a decrease in slow myosin content [46,92].

Comparing the changes of proportion of MyHC isoforms in the contractile apparatus during unloading among human subjects and experimental animals, we can see that they are similar in their direction and amplitude. In conclusion, the adaptation of the mammalian skeletal muscles to the unloading depends on the contractile and metabolic characteristics of skeletal muscle and is not dependent on the species of the mammal. As the certain connections exist between the contractile and metabolic characteristics of skeletal muscle it is understandable why the specific atrophy causes the decrease of the main function of the skeletal muscle.

## 6. Effect of reloading on the skeletal muscle

The plasticity of muscle is apparent in the phenomena such as muscle atrophy caused by inactivity, and recovery from atrophy. When atrophic muscles once again become active, the muscle mass and the volume reportedly increase in a relatively short period of time, but the recovery of the muscle strength takes much longer [93]. The recovery of motor activity after the hindlimb suspension is as fast as the recovery of the muscle strength. It is probably related with the regeneration of the muscle structures from disuse atrophy [89]. The fact that the increases in the muscular strength lag behind those in the muscular mass suggests the presence of functionally immature muscle fibres during the recovery process following disuse atrophy [89]. Several studies have shown that the increases in the muscle mass soon after reloading are attributable to oedema and do not actually represent recovery [89]. The recovery of the muscle mechanical properties depends also on the metabolism of the skeletal muscle. The two-week reloading period has shown that the Sol muscle metabolism can be restored [94]. Full recovery of slow-twitch muscle function via cross-sectional area and myonuclear domain size has been shown to need more time for restoration of neural and mechanical properties of muscle [81].

Disuse muscle atrophy can be experimentally induced by suspending animals by their tails [89,95], immobilizing joints, severing tendons or conducting muscle denervation [96]. Muscle atrophy in tail suspension is caused by hindlimb unloading, conserving the functions of nerves and joints. Reloading is thus possible after tail suspension and is suitable for investigating the recovery process following disuse muscle atrophy caused by sports injuries [89].

The reloading after hindlimb suspension shows that collagen III mRNA level at the end of the second week is higher than in control group. It has been found that in response to reload, the skeletal muscle expression of collagen I and III was markedly induced from the second day of reloading [4].

It has been shown that non-fibrillar type IV collagen mRNA level is decreasing in both, slow-twitch and fast-twitch muscles during the three weeks of hindlimb suspension, but two weeks of reloading period is obviously not enough to restore the metabolic states of this collagen in the basal lamina of the muscle fibre [26]. It was demonstrated that the reorganization of the basement membrane compounds needs certain time [97]. As type IV collagen plays a role in the regenerative process on ECM, including the matrix-associated receptors that underline muscle fibre-matrix interactions, it shows how complicated is the evaluation of the functional significance of type IV collagen metabolism [5].

The recovery of the collagen degradation markers during reloading period is different in slow- and fast-twitch skeletal muscles. MMP-2 level increased in slow-twitch soleus muscle during two weeks of reloading and in fast-twitch gastrocnemius muscle after one week reloading. The mRNA level of TIMP-1 increased in fast-twitch GM muscle after two weeks of reloading. A significant increase in mRNA level for MMP-2 was registered in Sol muscle during the reloading, showing that the reaction of MMP-2 on the pretranslational level is not fast in all muscles [52].

Concomitant to atrophy, numerous molecular events testify of a slow-to-fast transition of muscle properties [47,74]. The recovery of muscle properties effectively occurs on return to normal load [98]. It is also known that muscle fibre damage occurs during reloading, likely due to the inability of the muscle fibres to bear eccentric contractions and the consequent inflammation process [94,99]. Natural recovery seems to be most effective after reloading while several investigations show the delayed recovery of rats during running exercise [100].

The muscle tissue response to unloading seems to be more pronounced than the connective tissue response. The connective structures are protected from rapid changes in tissue mass, while muscle, which is known to act as a protein store for the organism, is subject to substantial and fast changes in tissue mass. However, it should be considered that important changes occur in the connective tissue structures during unloading despite the small changes in tissue mass.

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