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Review Article

# The mechanisms and treatments of muscular pathological changes in immobilization-induced joint contracture: A literature review

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ABSTRACT

The clinical treatment of joint contracture due to immobilization remains difficult. The pathological changes of muscle tissue caused by immobilization-induced joint contracture include disuse skeletal muscle atrophy and skeletal muscle tissue fibrosis. The proteolytic pathways involved in disuse muscle atrophy include the ubiquitin-proteasome-dependent pathway, caspase system pathway, matrix metalloproteinase pathway, $Ca^{2+}$-dependent pathway and autophagy-lysosomal pathway. The important biological processes involved in skeletal muscle fibrosis include intermuscular connective tissue thickening caused by transforming growth factor-β1 and an anaerobic environment within the skeletal muscle leading to the induction of hypoxia-inducible factor-1α. This article reviews the progress made in understanding the pathological processes involved in immobilization-induced muscle contracture and the currently available treatments. Understanding the mechanisms involved in immobilization-induced contracture of muscle tissue should facilitate the development of more effective treatment measures for the different mechanisms in the future.



## Introduction

Joint contracture is currently a common clinical disease that is characterized by the reduction of range of motion (ROM) in the active or passive state of the joint.[1–3] And it usually occurs in joint trauma, arthritis or central nervous system disease, but the most common cause is still joint immobilization.[2] As is known to us, joint immobilization is usually used as a crucial treatment for fractures, joint dislocations, and ligament injuries.[2,4] However, after a long period of immobilization to form joint contracture, the rehabilitation treatment is very difficult, even surgical treatment such as arthroscopic arthrolysis is still hard to restore the total ROM.[5] In addition to this, there are differences in the rate of progression of joint contracture caused by immobilization between different species.[6,7] Despite this, joint function can be restored by removing joint immobilization and reactivating. For example, flexing knee joint contracture that occurs in rats in 2 weeks can be completely restored, but it is difficult to restore completely when immobilization time over 4 weeks.[8] It is noteworthy that two different structural components make contribution to the development of joint contracture. On the one hand, myogenic contracture accounts for the main part in the early stage which caused by the changes of muscle, tendon, fascia, etc. On the other hand, arthrogenic contracture will be the principal component in the later stage which caused by the changes of bone, cartilage, joint capsule and ligament, etc.[9–12] Corresponding treatment of the mechanism of skeletal muscle changes can improve the symptoms of joint contracture, thereby improving the quality of life of patients and benefiting the reasonable distribution of social medical resources. Consequently, this article was intended to review the mechanisms and treatments of muscular pathological changes.

A search of the PubMed®, Embase® and Cochrane Library databases from 30 June 1980 to 30 June 2018 was undertaken using the combined search terms of “contracture” or “joint contracture” or “muscle contracture” to identify relevant articles that were subsequently screened by the authors.

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## Disuse skeletal muscle atrophy

Joint contracture is one of the common complications following continuous joint immobilization, in which disuse muscle atrophy occurs in the skeletal muscle. Firstly, the cross sectional area (CSA) of the muscle fiber was reduced and the length of the muscle fiber was shortened under the microscope.[13–15] No matter a classic plaster cast model in previous research, or an emerging fixing method in recent years, such as the hook-and-loop fastener immobilization of Onda et al.[16] and the spiral wire immobilization of Aihara et al.[4] They all found this phenomenon. Secondly, there was a phenomenon that the muscle cytoplasm was lightly stained and the number of interstitial and nucleus increased with the migration and aggregation of the nucleus.[13] This phenomenon indicated that the synthesis of muscle protein was weakened, and the proteolysis was enhanced.[13] Similar to other tissues, skeletal muscle tissue may contain at least five proteolytic pathways during immobilization-induced joint contracture, including ubiquitin-proteasome-dependent pathway, caspase system pathway, matrix metalloproteinase pathway, $Ca^{2+}$-dependent pathway, and autophagy-lysosomal pathway.

### *Ubiquitin-proteasome dependent pathway*

Recent evidence demonstrated that ubiquitin-proteasome-dependent proteolysis plays a key role in disuse skeletal muscle atrophy. For example, polyubiquitination involves the sequential action of the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin-protein ligases (E3).[17] The E1 enzyme has low level expression in skeletal muscle, and its mRNA level is not regulated in catabolic states.[17] A previous study reported that E1 is an extremely active enzyme capable of charging excess amounts of E2 with ubiquitin, and one E2 generally interacts with one or a limited number of E3 species that recognize specific protein substrates.[18] Although there are presumably as many as 1000 E3s in mammalian cells, only a very limited number of E3s that are upregulated in muscle atrophy have been identified.[18] Since the first identification of muscle-specific E3s, including muscle atrophy F-box protein (MAFbx or Atrogin-1) and muscle ring finger-1 protein (MuRF-1) in 2001, it was proved that MAFbx and MuRF-1 are both involved in the regulation of skeletal muscle atrophy under various pathological and physiological condition.[19] In a study which used a mouse model of knee joint contracture with selective gene knockout for the experimental group, the overexpression of MAFbx and MuRF-1 in the myotube after immobilization in the control group was shown to cause disuse skeletal muscle atrophy, but in contrast, the experimental group was resistant to disuse skeletal muscle atrophy.[19] In addition, a case report that described the treatment of an injury of the medial head of the gastrocnemius, also called tennis leg, in which knee joint contracture, ankle joint contracture and disuse atrophy of the gastrocnemius muscles occurred during immobilization therapy, demonstrated that MAFbx and MuRF-1 also played a key role in inducing disuse skeletal muscle atrophy.[20]

Another important point is that insulin-like growth factor 1 (IGF-1), phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) are the major signaling pathways for the activation of MAFbx and MuRF-1.[21–23] As early as 1999, it was proposed that the transcription factors including forkhead box O (FoxO), MAFbx, and MuRF-1 in the downstream signal of Akt promote muscle protein breakdown and muscle atrophy.[24] Activating Akt transfer from the cell membrane to the nucleus and phosphate FoxO.[24] The phosphorylated FoxO is transported out of the nucleus and loses control of the target gene.[24] When muscle atrophy occurs, PI3K and Akt pathways are inhibited and dephosphorylated FoxO is returned to the nucleus, promoting the transcription of MAFbx, MuRF-1, and some pro-apoptotic genes.[24] It is worth mentioning that a previous study demonstrated that the regulation of Akt and its downstream signaling pathways glycogen synthase kinase-3β (GSK-3β), mammalian target of rapamycin (mTOR) and FoxO is associated with skeletal muscle atrophy by measuring Akt and several of its downstream anabolic targets about GSK-3β, mTOR, p70S6 kinase (p70S6K), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and catabolic targets about FoxO1, FoxO3, MAFbx and MuRF1 in human quadriceps.[25] Prior to this, previous research had been suggested that Akt plays a key role in the regulation of skeletal muscle atrophy in rodents, but it had not been well proved in humans.[26]

There is also a key pathway for MuRF-1, namely inhibitor of nuclear factor kappa-B kinase (IKKβ/NF-κB). NF-κB regulates the expression of many cellular genes and is an important signal transcription factor in the process of disuse muscle atrophy.[27] A study was performed that the right limbs of the 16-week-old male mice were immobilized in the control group, while the proteasome inhibitor MG132 that inhibited ubiquitin-proteases was used in the treatment group.[28] The MG132 inhibited the expression of MAFbx and MuRF-1, and further inhibited the NF-κB pathway. Seven days later, the transcription level of MAFbx and MuRF-1 which was measured by qPCR and Western blot in the tibialis anterior muscle of the treatment groups was lower than that of the control groups. Furthermore, the CSA of the tibialis anterior muscle fibers in the treatment groups was larger than the control groups.[28] Overall, the proteasome inhibitor MG132 accelerated the recovery of disuse muscle atrophy after immobilization, and the clinical application was worth looking forward.

The ubiquitin-proteasome pathway ultimately hydrolyzes the protein through the 26S proteasome, where the 20S proteolytic core combines with the two 19S regulatory complexes to form the 26S proteasome.[17] In the course of disuse muscle atrophy after immobilization, the 26S proteasome in the ubiquitin-proteasome-dependent pathway is up-regulated.[17] In this pathway, a poly-ubiquitin protein chain is covalently attached to the substrate by a ubiquitin conjugation mechanism, and then the targeted protein is recognized and degraded by the 26S proteasome.[17] It is generally recognized that skeletal muscle proteasomes possess three major peptidase activities including chymotrypsin-like, trypsin-like or caspase-like activities.[17] For example, previous research which immobilized the left hind limbs of male Wistar rats found that the rate of ubiquitination and chymotrypsin-like activity were increased in the gastrocnemius muscle and soleus muscle during the immobilization period.[21] After a while, the rate of ubiquitination and chymotrypsin-like activity were gradually returned to normal during the recovery period.[21] In addition, previous research indicated that disuse muscle atrophy of the tibialis anterior muscle and gastrocnemius muscle was formed during knee joint immobilization, but ubiquitin-proteasome system was still activated during reactivation.[14,15] Furthermore, the chymotrypsin-like and trypsin-like activities associated with the ubiquitin-proteasome system remained increased and caused muscle proteolysis, which could not be fully reversed or even worsened.[14,15]

It is widely believed that two targets of muscle-specific E3s are eukaryotic translation initiation factor 3-f (eIF3-f) and myogenic differentiation (MyoD) which is one of the myogenic regulatory factors (MRFs).[29] This pathway is activated in atrophic muscle during both immobilization and recovery period.[29] As parts of the ubiquitin-proteasome targets, it is suggested that MRFs are closely related to the recovery of muscle atrophy.[22,29] In addition,

the common belief is that the regeneration process after skeletal muscle atrophy depends mainly on the myogenic satellite cells.[30] When skeletal muscle undergoes traction, movement or injury stimulation, they can activate the proliferation and differentiation of myogenic satellite cells into myoblasts.[30] After several cycles of proliferation, most of the myoblasts begin to differentiate and fuse to form new muscle fibers, or repair directly.[30] At the site, a few cells exit the cell cycle to self-renew.[30] When muscle satellite cells are activated, MRFs including MyoD, myogenic factor 5 (Myf5), myogenin, and myogenic regulatory factors 4 (MRF4) begin to perform precise and orderly regulation of a series of cellular changes such as proliferation and differentiation.[31–33] Specifically, MRFs bind to many enhancer regions of muscle-specific genes and regulate gene transcription to ameliorate muscle atrophy.[31–33] MyoD and Myf5 are markers of muscle satellite cell activation and proliferation.[30] Previous research indicated that the protein level of Myf5 was decreased during knee joint flexion immobilization of the male Wistar rats. Furthermore, it was associated with a significant increase in the number of apoptotic nuclei in the gastrocnemius muscle and soleus muscle.[21] For expected, the protein level of Myf5 was quickly returned to normal during recovery period. Myogenin and MRF4 are markers of muscle satellite cell differentiation, but little information has been attempted to search the change of Myogenin, MRF4 and MyoD in disuse muscle atrophy during immobilization-induced joint contracture.[30,34–36] Consequently, they are often used as indicators of the recovery after denervation-induced disuse muscle atrophy.[37]

*Caspase system pathway*

The caspase system pathway plays an important role in inducing apoptosis of myocytes. The caspase enzymes are rich in cysteine, and when they are activated, they can be cleaved at the specific aspartic acid residue of the target protein.[38] To illustrate this point, a previous study used the disuse muscle atrophy model by immobilized the left hind limbs of male Wistar rats. Then disuse muscle atrophy occurred in the left gastrocnemius muscle and the apoptotic body-associated caspase-3, caspase-8, and caspase-9 activity during immobilization and recovery period were measured to further research.[39] It was taken for granted that the activities of caspase-3, caspase-8 and caspase-9 showed that on the 8th day of immobilization, the above caspase activity was increased, indicating that the mitochondria-associated apoptosis pathway was activated. After 10 days, these indicators returned to normal, indicating that the occurrence of disuse muscle atrophy after immobilization was related to the activity of caspase. In previous research, the left hind limbs of female FVB/N mice were immobilized for 1, 2 and 3 weeks respectively, then reactivated for 1 week. The results showed that the activity of caspase-3 was gradually increased during the immobilization period.[40] After 2 and 3 weeks of immobilization, the activity of caspase-3 was 5 and 6 times respectively higher than that of the control group.[40] Although the activity of caspase-3 was gradually decreased after 1 week of reactivation, the level remained 3 times higher than that in the control group.[40] In addition, a previous study which used caspase-3 gene knockout mice found that caspase-3 deficiency significantly attenuated the decrease of gastrocnemius and soleus muscle mass after 2 weeks of plaster immobilization.[41] These findings indicated that caspase-3 played a key role in the induction of disuse muscle atrophy, which may be through the action of apoptosis and inflammatory pathways.[41] Therefore, this type of high-level caspase system-mediated apoptosis may be one of the reasons for disuse muscle atrophy in the immobilized phase and deterioration during recovery.

*Matrix metalloproteinase pathway*

Matrix metalloproteinases (MMPs) are extracellular zinc-dependent endopeptidases which can be divided into gelatinases, matrix degraders, matrix lysins, furin-activated MMPs, and other secreted MMPs according to their different domains and substrates.[42] In the course of disuse muscle atrophy caused by immobilization-induced joint contracture, MMP-2 plays a major role.[43] Previous research revealed that MMP-2 and MMP-14 mRNA levels in the tibialis anterior muscle were unchanged at day 8 of immobilization, but the levels were increased at day 1 of recovery compared with day 0 of immobilization, then were gradually returned to the basal values at day 10 of recovery.[15] Interestingly, the mRNA levels of tissue inhibitor of metalloproteinase (TIMP) −1 and −2 in the tibialis anterior muscle were increased by ~30- and ~2-fold at day 8 of immobilization respectively and remained elevated at the day 1 of recovery, then returned to the basal values at day 6 of recovery.[15] Therefore, the MMP pathway may play a role in the development of muscle atrophy during remobilization after joint fixation.

*$Ca^{2+}$ dependent pathway and autophagy-lysosomal pathway*

At present, the study of the role of $Ca^{2+}$-dependent pathways in the development of disuse skeletal muscle atrophy in joint contracture remains limited. It is generally accepted that the calpain system is synergistic with the ubiquitin-proteasome-dependent system in degrading myofibrillar protein.[44] Similarly, lysosomal and ubiquitin-proteasome systems may also act synergistically to degrade certain protein substrates. The lysosomal cathepsin does not degrade myofibrillar proteins. Its main function is to degrade membrane proteins, including receptors, ligands, and channel and transport proteins. Just as the microtubule-associated protein light chain 3 (LC3) is used widely to monitor the autophagy-lysosome system, the cytosolic form of LC3 (LC3-I) conjugates with phosphatidylethanolamine to form an LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes.[45] A study of the preventive effect of dietary intake of medium-chain fatty acid triglyceride on immobilized muscular atrophy in rats measured the levels of LC3-I and LC3-II in the soleus muscle as the amount of LC3-II is correlated with the extent of autophagosome formation and an increased LC3-II/LC3-I ratio is representative of accelerated autophagy-lysosome activity.[45] Unilateral immobilization increased LC3-II levels, but not LC3-I levels, resulting in a higher LC3-II/LC3-I ratio in the immobilized muscle.[45] Therefore, the immobilized joint contracture caused by disuse muscle atrophy was associated with the lysosomal pathway.[45] In addition, the results of a previous study also demonstrated that after large initial remodelling to restore muscle mass and muscle fibre integrity, such as by eliminating defective organelles, autophagy-lysosomal phagocytosis may be induced in the reactivated tibialis anterior muscle.[14]

## Skeletal muscle fibrosis

In addition to disuse muscle atrophy, skeletal muscle tissue fibrosis is also another pathological change that causes muscle tissue during joint contracture. Its main manifestation is the overexpression and accumulation of collagen in the connective tissue of the muscle that including the perimysium, endometrium and epithelium. Skeletal muscle tissue fibrosis causes organ dysfunction, such as a decrease in skeletal muscle extensibility, which is thought to be associated with the degree of limitation of ROM.

In a previous study, the rat ankle joint was fixed in a fully inflexed position, allowing the soleus to be fixed in a shortened

position.[46] They used the slope value/muscle physiological cross-sectional area (PCSA) ratio to express skeletal muscle extensibility.[46] After 4, 8, and 12 weeks of immobilization, the slope value/PCSA ratio of soleus was significantly higher than that at 1 and 2 weeks, and the similar result was also observed for the degree of soleus endometrium thickening.[46] Besides, there was a significant difference in type I collagen expression in the soleus endometrium at 4, 8, and 12 weeks relative to that at 1 and 2 weeks.[46] Therefore, overexpression of type I collagen led to endometrium thickening of soleus, and these pathological changes indicated the presence of immobilization-induced muscle fibrosis in soleus.[46] Furthermore, the investigators in previous study also showed that skeletal muscle fibrosis in rats increased in a time-dependent manner within 4 weeks of immobilization.[47] Similarly, another experimental study that used of cyclic muscle twitch contraction to inhibit immobilization-induced muscle contracture and fibrosis in rats also found the similar conclusion.[48] The results showed that the expression of type I and type III collagen in the soleus perimysium of the immobilization group was significantly higher than that of the control group, and the expression of type I collagen in the soleus endometrium of the immobilization group was also significantly higher than that of the control group.[48]

Equally important of the increase in collagen, skeletal muscle extensibility depends not only on intramolecular and intermolecular cross-linking, but also on the arrangement of fibrin.[13] A previous study investigated that the muscle contracture was affected by the shortening muscle fibers during the early stage of immobilization, after which the collagen adapted by the fibril arrangement in the endomysium becoming more circumferential, but the degree of ROM was further reduced.[13]

A previous study investigated the biological processes involved in immobilization-induced skeletal muscle fibrosis by comparing the soleus muscles of rats that were divided into immobilized and control groups.[47] The results showed that the expression of protein and mRNA levels of α-smooth muscle actin (α-SMA), interleukin -1β (IL-1β) and transforming growth factor-β1 (TGF-β1) mRNA, type I collagen and type III collagen in the immobilization groups were significantly higher than the control groups at all due time.[47] In the early stage of immobilization, upregulation of IL-1β and TGF-β1 by macrophage may promote fibroblast differentiation and affect muscle contracture.[47] In addition, the expression of protein and mRNA level of hypoxia inducible factor-1α (HIF-1α) were significantly higher in the immobilization group compared with the control group at the end of 4 weeks.[47] Furthermore, the expression of protein and mRNA level of HIF-1α, α-SMA and type I collagen were significantly higher at the end of 4 weeks compared with that at the end of 1 week and 2 weeks in the immobilization group.[47] This study revealed a biological process of immobilization-induced skeletal muscle fibrosis, suggesting that other biological processes of potential fixation-induced skeletal muscle fibrosis were skeletal muscle hypoxia.[47]

## Related treatment progress

In addition to the aforementioned inhibition of the ubiquitin-proteasome dependent pathway by the proteasome inhibitor MG132 to slow the rate of skeletal muscle protein hydrolysis, long-term regular exercise can also minimize the negative effects of muscle tissue changes.[28] During training, by preserving new nuclei that develop in muscle tissue, prior strength training induces muscle hypertrophy and protects the structural and mechanical properties of skeletal muscle during disuse due to immobilization.[28] Therefore, physical training load before immobilization may affect muscle changes during immobilization.[28] A two-week rehabilitation programmed (swimming five times per week, 60 min each time) before the left hind limbs of female rats were immobilized for 5 days to induce degenerative atrophy of the white calf and soleus muscles demonstrated that limb immobilization significantly reduced the cross-sectional area of the white gastrocnemius muscle, but no changes were observed in the soleus muscles (mainly composed of type I fibers) of the swim-trained animals.[49] This result showed that prior exercise can reduce hypotrophy of skeletal muscle composed of type I fibers after immobilization.

Muscle tissue needs tension to maintain and restore mechanical properties.[50] When the tension is reduced, for example, after immobilization, the mechanical properties are weakened, and contracture of the joint is induced.[48] The use of stretching to treat joint contracture is a safe and effective treatment.[51] A previous study comparing stretch of variable torque and duration found low torque-long duration stretch was most effective in increasing passive range of motion in the contracted knees of laboratory rats.[51] The stretching procedure provided more tensile load than during four-legged walking and might induce increased migration of fibroblasts and increased collagen synthesis at the bone-capsular attachment site.[51] Furthermore, low torque-long duration stretching was also effective in the recovery of some of the mechanical properties such as phase lag, peak maximum load, and deformation.[51] A study that used a spring balancer set at different moments to stretch in the direction of dorsiflexion after fixing the ankle joint in rats 2 weeks concluded that stretching can improve the ROM and increase the flexibility of the soleus muscle.[50] However, after a 4-week period of immobilization, short-term stretching was not sufficient to improve the flexibility of the soleus muscle and not enough to ameliorate its morphological and physiological characteristics.[50] This study further demonstrated the importance of small torque and long-term continuous stretching for improving the mechanical properties and joint mobility of muscle tissue. The current clinical application of this type of exercise treatment includes the use of continuous passive motion training devices and neuromuscular electrical stimulation function training devices.[48]

Oxidative stress and inflammatory reactions increase in immobilized skeletal muscle tissue, and heat stimulation can prevent immobilization-induced disuse muscle atrophy and myogenic contracture.[52–54] A previous study indicated that heat stimulation provided protection by against oxidative stress and preserved muscle mass.[52] Apart from the heat stimulation, there was also a significant effect in inhibiting the deterioration of muscle tissue during joint contracture by some certain drugs. Curcumin exhibited antioxidant and anti-inflammatory properties that can prevent the activation of animal proteasomes and promoted skeletal muscle regeneration.[53] A study also showed that celecoxib feed can partially reduce myogenic contracture after immobilization, and these results meant that inflammation and nociception were also involved in the formation of myogenic contracture.[54] Inhibition of the inflammatory response can relieve muscle tissue fibrosis to some extent. In addition to drugs, certain physical factor treatments such as low intensity pulsed ultrasound and infrared also performed a preventive effect on joint contracture.[55,56] These treatments reduced adhesions, fibrosis, inflammation and hypoxia response after immobilization of the joint to varying degrees, thereby extenuating joint dysfunction.

## Conclusion

Changes in musculature caused by immobilization-induced joint contracture include disuse skeletal muscle atrophy and skeletal muscle fibrosis. Understanding the mechanism of immobilization-induced contracture of muscle tissue and finding the most appropriate treatment measures for different mechanisms should be the goals of future research.

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## Ethical statement

Ethics approval and consent to participate are not applicable.

## Conflicts of interest

There are no conflicts of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cjtee.2019.02.001.

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