Sports Medicine and Health Science 7 (2025) 173–184

Contents lists available at ScienceDirect

Sports Medicine and Health Science

journal homepage: www.keaipublishing.com/smhs

Review

Mechanisms of muscle repair after peripheral nerve injury by electrical stimulation combined with blood flow restriction training

Xiaolei Chu [a,1], Jiaojiao Sun [b,1], Jiajia Liang [b], Wenjie Liu [b], Zheng Xing [a], Qi Li [a,*], Qingwen Li [b,**]

[a] Department of Rehabilitation, Tianjin University Tianjin Hospital, Tianjin, China
[b] Tianjin Key, Laboratory of Exercise Physiology and Sports Medicine, Institute of Sport, Exercise and Health, Tianjin University of Sport, Tianjin, China

ARTICLE INFO

Keywords:
Electrical stimulation
Blood flow restriction
Muscle fibers
mTOR
Muscle protein synthesis

ABSTRACT

This review elucidates the impact of electrical stimulation (ES) and blood flow restriction (BFR) training on muscle function. ES induces a transformation in muscle fibers type by rearranging myosin heavy chain isoform patterns. Additionally, it influences muscle protein synthesis and degradation through specific signaling pathways such as protein kinase B/mechanistic target of rapamycin (Akt/mTOR), as well as via autophagy and the ubiquitin-proteasome system, thereby effectively maintaining muscle mass. BFR, on the other hand, restricts muscle blood flow, leading to metabolic products accumulation and localized hypoxia, which not only promotes the recruitment of fast-twitch fibers but also activates the mTOR signaling pathway, enhancing muscle protein synthesis. The combination of ES and BFR synergistically facilitates muscle protein synthesis through the mTOR pathway, thereby accelerating the recovery of muscle function following peripheral nerve injury.

1. Introduction

According to relevant research, up to one million individuals worldwide suffer from peripheral nerve injuries (PNI) annually. In the United States, 2.3% of patients with limb trauma are diagnosed with one or more PNIs.[1] In China, the number of PNI cases ranges from 300 000 to 500 000, accounting for 2.8% of trauma patients.[2] Following a PNI, patients may face a myriad of complex physiological and functional challenges, including, but not limited to, sensory loss, pain, muscle weakness and atrophy, and impaired motor coordination. The interruption of nerve signal transmission caused by the injury not only impairs normal muscle function and strength but can also lead to prolonged sensory deficits and muscle atrophy. This condition poses a direct challenge to patients' ability to perform daily activities, affecting their self-care and social participation, and places a significant burden on families and society.[2] Therefore, timely and effective interventions are crucial to aid patient recovery.

In this context, the restoration of muscle function emerges as a pivotal need in the rehabilitation process of patients with PNI. Specialized exercise regimens and physical therapy interventions can gradually help patients regain strength and functionality in the affected muscles. This process is crucial for restoring patients' self-care capabilities, enhancing their quality of life, and mitigating long-term sequelae. Moreover, by slowing muscle atrophy and improving muscle strength, these interventions boost patients' confidence in their recovery progress, promoting psychological well-being and social adaptation. In the fields of sports medicine and rehabilitation, enhancing muscle performance is a key factor in improving athletic performance, facilitating recovery, and maintaining overall health.

Recent scientific research has unveiled various effective muscle enhancement strategies, among which Blood Flow Restriction (BFR) training and Electrical Stimulation (ES) have garnered widespread attention and application due to their unique mechanisms of action and ease of use.[3–5] The aim of this review is to explore the unique mechanism of action of electrical stimulation therapy and blood flow restriction training on each of the muscle fiber structure and muscle protein metabolism, and then to speculate how these two methods synergistically contribute to the process of muscle strength restoration after peripheral nerve injuries when applied in combination. In this comprehensive analysis, we expect to explore a more efficient treatment plan for muscle strength reconstruction for patients with peripheral nerve injuries to help them recover motor function and improve their quality of life.

* Corresponding author. Department of Rehabilitation, Tianjin University Tianjin Hospital, Tianjin, China.
** Corresponding author. Tianjin Key, Laboratory of Exercise Physiology and Sports Medicine, Institute of Sport, Exercise and Health, Tianjin University of Sport, Tianjin, China.
E-mail addresses: Liqi_82@126.com (Q. Li), leeqw1101@163.com (Q. Li).
[1] These authors have contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.smhs.2024.10.002
Received 7 June 2024; Received in revised form 20 September 2024; Accepted 9 October 2024
Available online 11 October 2024

Abbreviations

4E-BP1	eukaryotic initiation factor 4E binding protein 1
Akt(PKB)	protein kinase B
BFR	Blood Flow Restriction
ES	Electrical Stimulation
FT	fast-twitch muscle fibers
GH	Growth Hormone
IGF-1	Insulin-like growth factor-1
MPB	Muscle Protein Breakdown
MPS	Muscle protein synthesis
mTOR	mechanistic target of rapamycin
mTORC1	mTOR Complex 1
mTORC2	mTOR Complex 2
PI3K	phosphatidylinositol 3-kinase
PNI	peripheral nerve injury
S6K	ribosomal protein S6 Kinase

2. Physiological changes following peripheral nerve injury

Muscle atrophy and strength loss following PNI is a complex biological process involving multiple factors. After peripheral nerve injury, the decline in muscle strength presents a multidimensional character, which is not only reflected in the weakening of muscle contraction force, but also affects the performance of muscle endurance. Specifically, type I muscle fibers (i.e., slow muscle fibers) are more resistant to fatigue and play a central role in prolonged, low-intensity muscle activities. Their ability to provide a steady supply of energy over prolonged periods of activity ensures that muscle strength remains relatively stable over long periods of time, especially when the body has to overcome gravity to maintain a particular posture for a prolonged period of time. In contrast, type II muscle fibers (fast muscle fibers), especially type 2A (IIA) and type 2X (IIX), have stronger contraction forces and faster contraction rates. They can be rapidly activated in a short period of time to produce a significant muscle contraction effect. However, after peripheral nerve injury, muscle strength decreases due to the impairment of muscle fiber type-specific function and its disturbed synergy with each other, a process that not only affects the performance of a single muscle fiber type, but also disrupts the overall coordination of the muscle fiber network, leading to an overall decline of the muscle in both the dimensions of contraction force and endurance. Previous studies have shown that PNI affects muscle fibers and muscle protein metabolism, leading to changes that impact muscle tissue. These changes are not confined to the local muscle area but can extend to entire muscle groups, consequently affecting the individual's overall motor abilities and quality of life.

2.1. Changes in muscle fibers type

Following PNI, the conduction of nerve signals is impaired, leading to a reduction in neural stimulation received by the muscles. Muscle fibers depend on these neural signals for normal contraction and relaxation. The lack of nerve signals results in structural changes in muscle fibers, primarily manifesting as atrophy and fibers type transformation. This, in turn, affects muscle strength output and endurance.

Skeletal muscle is an exceedingly complex tissue composed of various types and subtypes of fast and slow fibers. Muscle fibers can be classified based on tissue color, fibers diameter, contraction time, power output, usage in aerobic and anaerobic activities, fatigue resistance, capillary density, predominant oxidative and glycolytic energy metabolism, mitochondrial density, myoglobin levels, glycogen levels, triglyceride storage, and the presence of the phosphocreatine system.[6] Muscle fibers also adjust their phenotypic characteristics in response to changes in functional demands.[7] Myosin was first extracted from muscle tissue by Kühne, who named it based on its solubility characteristics and its pronounced susceptibility to denaturation(Fig. 1). The primary distinction between muscle fibers types lies in their myosin complement, specifically the subtypes of myosin light and heavy chains.[7] One of the most useful molecular schemes for fibers typing is based on the distribution of highly abundant myosin heavy chain (MyHC) isoforms within individual muscle fibers.[8] In the adult skeletal muscle of small mammals, four MyHC isoforms can be expressed: MyHC-I (or slow type), and MyHC-IIa, MyHC-IIx, and MyHC-IIb (or fast types). However, in humans, although the MyHC-IIb gene exists, it is not expressed in adult muscle.[9,10] Human muscles contain three types of MyHC isoforms: type I, type IIa, and type IIx.

In the natural process of biological adaptation, the composition of muscle fibers undergoes alterations that can be observed during muscle development, maturation, nutritional changes, exercise-induced modifications, and natural aging.[11–14] Under certain conditions, the expression of myosin heavy chain (MHC) subtypes can shift from fast to slow isoforms or vice versa. Graded responses that induce muscle transformation can occur during immobilization, prolonged bed rest, denervation, resistance training, endurance exercise, and chronic electrical stimulation.[15] For instance, Moriggi's et al. research demonstrated that in both the vastus lateralis and soleus muscles of an immobilized (microgravity model) group,[16] there was an increase in type I and a decrease in type IIA MHC distribution. Other studies have indicated that prolonged bed rest results in a reduction of MyHC-IIa and an increase in MyHC-IIx in the soleus muscle, as well as a decrease in MyHC-I and an increase in MyHC-IIa in the vastus lateralis muscle.[8] Consequently, factors such as restricted activity, prolonged bed rest, and reduced physical activity due to PNI can directly lead to alterations in muscle fibers structure and function. These changes ultimately affect muscle strength and endurance, diminishing muscle contraction capacity and leading to a decline in overall muscle performance.

2.2. Alterations in muscle protein metabolism

Prolonged skeletal muscle unloading due to immobilization, nerve compression, denervation, microgravity, or extended bed rest following PNI exerts profound effects on skeletal muscle.[17] Post-PNI denervation leads to a rapid and progressive loss of muscle size and function, which some studies attribute to disrupted protein homeostasis. Following nerve injury, the rate of muscle protein synthesis (MPS) declines, while muscle protein breakdown (MPB) may increase. This imbalance between synthesis and degradation results in a net loss of muscle mass (muscle atrophy).[18–21] Research by Jokl and Konstadt demonstrated that muscle weight decreases after limb immobilization,[22] along with a reduction in both the total content and concentration of myofibrillar proteins (the contractile proteins of muscle tissue). Additional studies have shown that within the first 6 h of limb immobilization, the rate of protein synthesis in the immobilized muscles decreases[23]; furthermore, these studies revealed that after several weeks of immobilization, muscles

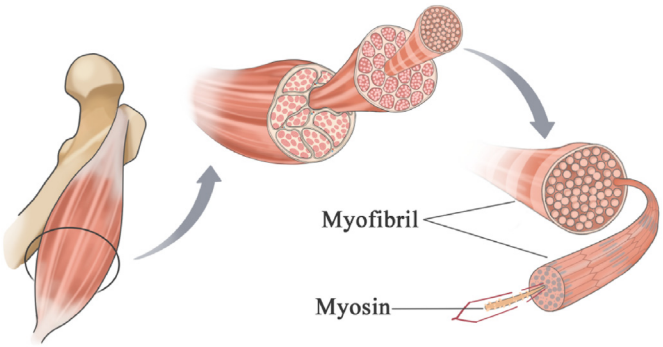

Fig. 1. The location of myosin in muscle and myogenic fibres.

predominantly composed of slow-twitch fibers exhibited characteristics of fast-twitch fibers, indicating a shift in muscle fibers type. This suggests that the increased levels of protein degradation and decreased rates of protein synthesis are accompanied by changes in bioenergetics and a trend toward the transformation of slow-twitch to fast-twitch muscle fibers.[16]

Recent studies have elucidated the molecular mechanisms underlying the imbalance between pathways that control protein synthesis and degradation.[17,20] Among these, several have identified a series of complex signaling pathways whose activation appears to be associated with enhanced muscle protein synthesis (MPS) and subsequent protein accumulation within muscle fibers. The most renowned of these pathways is the mechanistic target of rapamycin (mTOR) signaling pathway. The mTOR pathway can be activated by various stimuli, including mechanical load, growth factors such as insulin and IGF-1, and amino acids (particularly leucine).[24] Once activated, the mTOR pathway promotes MPS within muscle cells, thereby facilitating muscle fibers growth and repair.

mTOR exerts its effects through two complexes, mTORC1 and mTORC2, both of which play distinct roles in cellular metabolism and growth. Notably, mTORC1 is highly responsive to resistance training, which effectively activates mTORC1, leading to increased MPS and directly correlating with gains in muscle mass and strength.Its activation is crucial for ribosome biogenesis and protein translation,[25] processes indispensable for muscle adaptation and hypertrophy.[26] In the context of MPS, the mTOR pathway primarily functions through two downstream effectors—S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4E-BP1). Activation of S6K1 promotes ribosome biogenesis and mRNA translation, thereby increasing the synthesis of specific proteins, such as actin and myosin, which are vital for muscle contraction and strength development. Meanwhile, the phosphorylation of 4E-BP1 alleviates its inhibitory effect on the initiation factor eIF4E, enhancing cap-dependent translation initiation of mRNA, and further augmenting MPS.

While mTORC1 primarily stimulates MPS, it also indirectly influences muscle protein breakdown (MPB). Activation of mTORC1 inhibits the transcription of genes involved in autophagy and the ubiquitin-proteasome pathway.[27] When mTORC1 is activated, it suppresses the formation of the ULK1 complex, thereby blocking the initiation of the autophagy pathway and reducing MPB.[28] Autophagy and the ubiquitin-proteasome pathway are the two principal systems for protein degradation within muscle cells. Thus, by promoting MPS and concurrently inhibiting MPB, mTORC1 facilitates muscle hypertrophy.

Although mTORC1 is widely recognized as a signaling molecule that regulates muscle protein synthesis, mTORC2 also plays a role in promoting muscle growth.[29,30] mTORC2 can regulate protein synthesis through the modulation of the transcription factor c-Myc.[31] Specifically, MYC, as a direct regulator of ribosome biogenesis, can precisely control the transcription process of ribosomal RNA and its protein components, which in turn coordinates the processing of ribosomal RNA, the nuclear export of ribosomal subunits, and the expression of a series of gene products necessary for the initiation of mRNA translation,[32] and thus regulates the efficiency of protein synthesis. In addition, mTORC2 exhibits maintenance of a pool of muscle satellite cells,[33] a function that helps support long-term and sustainable muscle growth.

In conclusion, mTOR comprehensively regulates protein metabolic processes in muscle through a series of complex and fine-grained pathways. It not only promotes protein synthesis, but also inhibits protein catabolism, and this combined effect is crucial for repairing damaged muscles, enhancing muscle strength, and improving overall muscle health.

3. The effects of electrical stimulation on muscle tissue

3.1. Inducing transformation in muscle fibers types

Electrical stimulation (ES), as an effective biological intervention technique, has been demonstrated to alter myosin types and subsequently promote muscle fiber type transformation by regulating RNA transcription and translation processes. Various researchers have supported this view through different experiments (Table 1).

In studies conducted on rats, Termin et al. discovered that chronic electrical stimulation of fast-twitch muscles induces a reorganization of myosin heavy chain (MHC) isoform patterns, with a gradual reduction in HCIIb and a concomitant increase in HCIIa and HCIId.[43] The temporal progression and extent of these transitions suggest that HCIId serves as an intermediate form between HCIIb and HCIIa. Additional research involving chronic 10 Hz stimulation of rat fast-twitch muscles revealed rapid and reversible changes in the mRNA isoforms of myosin heavy chain within the tissue[34]: specifically, a swift decrease in HCIIb mRNA and a gradual increase in HCIIa mRNA. Upon cessation of stimulation for one day, HCIIb mRNA re-emerged, gradually increasing in a compensatory manner while being replaced by HCIIa mRNA. Wehrle et al. established myotube cultures from satellite cells of rat muscles composed of three different fiber types—slow-twitch soleus,[35] diaphragm, and fast-twitch tibialis anterior muscles—and subjected them to electrical stimulation. The findings indicated that prolonged electrical stimulation precipitated isoform switching. Furthermore, a study involving long-term electrical stimulation of denervated (sciatic nerve transection) fast extensor digitorum longus (EDL) muscle in adult rats over a span of four months corroborated that electrical stimulation induces muscle fibers type transitions.[36] Windisch et al. observed that within the first three weeks of stimulation, all IIb fibers and nearly all IIx fibers were replaced by IIa and I fibers, increasing to approximately 75% and 15%, respectively (normal extensor digitorum longus contains on average 45% IIb, 29% IIx, 23% IIa, and 3% I fibers). The proportion of IIa fibers remained stable at 75% for nearly two months before gradually being replaced by I fibers over the subsequent two months, following a sequential transition pattern of IIb/IIx→ IIa → I.

Similar changes to those observed in rats have also been noted in studies on rabbits. Mabuchi et al. discovered that prolonged intermittent stimulation (10 Hz, 8 h/d, 7 weeks) of the rabbit's fast-twitch tibialis anterior muscle resulted in the conversion of IIb fibers to IIa fibers.[37] Subsequently, Aigner and Pette demonstrated that the fast-to-slow fibers type transition follows a sequential order[44]: pre-existing IIa fibers are

Table 1

research target	author	electrical stimulation	parameters	Myofibre changes
rat	Kirschbaum et al.[34]	chronic electrical stimulation	10 Hz; 10 h/d	IIb→IIa
	Wehrle et al.[35]	chronic electrical stimulation	250 ms 40 Hz; 4 s/repeat	→I
	Windisch et al.[36]	chronic electrical stimulation	20 s/20 Hz; last 10 s	IIb/ IIx→IIa→I
rabbit	Mabuchi et al.[37]	Long-term intermittent stimulation	10 Hz; 8 h/d	IIb→IIa
	Leeuw, Pette[38]	low-frequency electrical stimulation	10 Hz (single pulse width 0.15 ms)/1 h; 12 h/d	IId→IIa→I
	Brownson et al.[39]	self-contained miniature stimulator	10 Hz; 3 w	IIb→I
patient	Andersen et al.[40]	functional electrical stimulation	60 Hz; 30 min/d	IIb→IIa
	Nuhr et al.[41]	low-frequency stimulation	15 Hz; 4 h/d	IId→I
	Gondin et al.[42]	neuromuscular electrical stimulation	75 Hz; last 400 s	IIx→IIa→I

Various scholars' studies on different subjects and changes in muscle fibre types induced by electrical stimulation. Abbreviations: h, hours; s, seconds; min, minutes; d, days, ms, milliseconds; Hz, hertz; w, watts.

converted first, while IIb and IId fibers must initially reach the IIa state before further conversion. Leeuw and Pette corroborated this finding, tracking the changes in myosin heavy chain (MHC) during the fast-to-slow transition in the rabbit tibialis anterior muscle under low-frequency stimulation.[38] It was observed that the predominant fast myosin isoform, HCIId, was replaced by HCIIa, which eventually was substituted by the slow myosin isoform, HCI. Kirschbaum et al. also concluded that electrical stimulation induces a transition from fast to slow myosin light and heavy chains at the mRNA level in rabbit fast-twitch muscles.[45] Another study involved continuous 10 Hz electrical stimulation for up to 3 weeks, followed by a recovery period of 12 days within the first 6 weeks after cessation of stimulation.[39] During the early stages of response to stimulation, fast MHC mRNA was replaced by slow MHC mRNA in the fast-twitch muscles of rabbits, specifically in the tibialis anterior and extensor digitorum longus muscles.

In human studies, similar adjustments in myosin expression patterns have been observed, suggesting the universal efficacy of electrical stimulation across species. Andersen et al. conducted long-term functional electrical stimulation (FES) training on the vastus lateralis muscle of spinal cord injury patients and found that functional electrical stimulation led to significant changes in myosin heavy chain (MHC) expression[40]: the expression levels of MHC IIa and MHC IIb shifted from being roughly equal to a state where MHC IIa predominated almost entirely. Nuhr et al. investigated the effects of chronic low-frequency stimulation (CLFS) on the quadriceps and hamstrings of healthy volunteers and observed that chronic low-frequency stimulation induced a shift in MHC isoform patterns from fast to slow,[41] with a roughly 20% decrease in the relative concentration of MHCIId/x and a 10% increase in the relative concentration of MHCI. The following year, Nuhr et al.'s subsequent study further confirmed that chronic low-frequency stimulation promotes the transition of MHC isoforms towards a slower phenotype, increasing MHCI expression.[46] Abdellaoui et al.'s research on the quadriceps of hospitalized COPD patients post-exacerbation revealed that after six weeks of neuromuscular electrical stimulation (NMES),[47] the proportion of type I fibers significantly increased in the neuromuscular electrical stimulation group. Gondin et al.'s study on isometric NMES training of the quadriceps demonstrated that after stimulation, both the regularly exercised and sedentary groups exhibited a notable transition from MHC-2x to MHC-2a and MHC-1, indicating a fast-to-slow shift.[42] Both groups experienced increases in maximal voluntary contraction force and neural activation post-NMES (approximately + 30% and + 10%, respectively), with significant muscle hypertrophy observed in both type I and type II fibers. Toth et al. investigated whether NMES could serve as an alternative to conventional exercise for breast cancer patients and found that NMES promoted muscle fiber hypertrophy, particularly in MHC IIa fibers, and tended to induce a fibers type transition in MHC II fibers.[48] However, NMES had minimal impact on the contractile strength of single muscle fibers and could not prevent the functional decline of MHC IIa fibers. In the same year, Toth et al. also studied the quadriceps after anterior cruciate ligament (ACL) injury and reconstruction, finding that early use of NMES reduced skeletal muscle fiber atrophy in MHC II fibers.[49] NMES preserved the contractility of MHC I fibers, increased maximal contraction velocity, and maintained power output (but not in MHC II fibers). Six months post-surgery, no significant difference in overall muscle strength was observed between the NMES and non-NMES groups. These findings collectively demonstrate that NMES can influence muscle fiber type transitions and enhance muscle function by affecting muscle fibers.

In summary, electrical stimulation (ES) can induce a reduction in IIb/IIx fibers, an increase in IIa fibers, and a conversion between IIa and I fibers (Fig. 2). As an effective method for regulating muscle fibers type transitions, ES has demonstrated significant effects in animal models and shows substantial potential for improving muscular performance in humans.

3.2. *Regulation of muscle protein synthesis and degradation*

Electrical stimulation (ES), as a non-invasive therapeutic method, can mimic neural signals to induce muscle contractions, thereby enhancing muscle strength and endurance. This is particularly beneficial for individuals experiencing muscle function decline due to injury or disease. Research indicates that ES can also enhance muscle protein synthesis (MPS) rates or slow muscle protein breakdown (MPB) by activating intracellular signaling pathways within muscle cells, thereby aiding in muscle growth and recovery.

Akt, also known as protein kinase B (PKB), is a serine/threonine-specific protein kinase that plays a pivotal role in various cellular processes, including protein synthesis, glucose metabolism, apoptosis, cell proliferation, and other cellular functions. The activation of mTOR complex 1 (mTORC1) stimulates downstream effectors such as S6 kinase (S6K) and 4E-binding protein 1 (4E-BP1), which directly promote protein synthesis. Previous studies have indicated that Akt and p70 S6 kinase (p70S6K) are crucial in the intracellular signaling integration of protein synthesis in skeletal muscle cells.[50–53] Akt has been shown to be activated by mechanical stretching, which induces muscle hypertrophy.[51,53–55] Additionally, Akt can indirectly regulate p70S6K by activating the mTOR pathway.[56] The upregulation of phosphorylated p70S6K (p-p70S6K) is associated with increased muscle protein synthesis (MPS), as it enhances mRNA translation by phosphorylating ribosomal protein components.[57] Increased phosphorylation of Akt (p-Akt) and p-p70S6K can be observed during muscle hypertrophy and the regeneration of atrophied skeletal muscle(Fig. 4).[58,59]

Ohno et al. investigated the effects of microcurrent electrical neuromuscular stimulation (MENS) on the regeneration of atrophied skeletal muscle in rats subjected to hindlimb suspension.[60] The study found a reduction in both the mass and protein content of the soleus muscle post-suspension. However, microcurrent electrical neuromuscular stimulation intervention in the rats resulted in a faster recovery of the

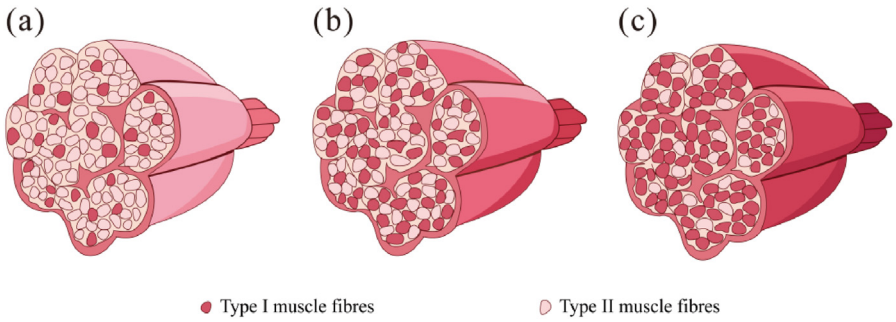

Fig. 2. Cross-sectional view of a muscle.
(a) Type IIb/IIx muscle fibres; (b) Type IIa muscle fibres; (c) Type I muscle fibres
Electrical stimulation induces the conversion of type II muscle fibres to type I, and type IIa may be an intermediate form in the conversion of type II to type I.

atrophied soleus muscle compared to the natural recovery group, with a significant increase in the phosphorylation levels of p70 S6 kinase and protein kinase B (Akt) in the microcurrent electrical neuromuscular stimulation group. This suggests that microcurrent electrical neuromuscular stimulation may serve as a potential extracellular stimulus capable of activating intracellular signaling involved in muscle protein synthesis (MPS), thereby promoting muscle recovery. Marlou L and colleagues conducted a study on comatose ICU patients under complete sedation, using neuromuscular electrical stimulation (NMES) to determine its efficacy in mitigating skeletal muscle atrophy.[61] The results showed that the NMES group exhibited higher expression levels of MAFBx, MuRF1, FOXO1, mTOR, and P70S6K compared to healthy controls. NMES also reversed the decline in mTOR phosphorylation, thereby increasing the rate of MPS, consistent with previous findings by Wall et al.[62] Mettler et al.'s research demonstrated that acute NMES increased the phosphorylation levels of mTOR and S6K1 in the vastus lateralis muscle of the paretic leg in stroke patients, with a marked increase in the anabolic response of phosphorylated muscle.[63] This indicates that NMES may help maintain the capacity for MPS in paralyzed muscles of chronic stroke patients. Khodabukus et al. utilized an intermittent stimulation protocol to apply electrical stimulation (ES) to 3D tissue-engineered human muscle bundles.[64] The study found that ES significantly enhanced mTORC1 activity in a frequency-dependent manner and increased the activation of anabolic pathways involving mTOR, S6K, Akt, and ERK1/2. These studies collectively confirm that ES can promote MPS through the activation of the Akt/mTOR signaling pathway.

Moreover, electrical stimulation (ES) can reduce muscle protein breakdown (MPB) by inhibiting the ubiquitin-proteasome pathway, the primary intracellular mechanism for protein degradation. This pathway regulates the total protein content by marking and degrading damaged or unnecessary proteins. By slowing down protein degradation, ES helps maintain or increase muscle mass. Liu et al. immobilized the knees of rabbits and found that after four weeks,[65] the levels of five autophagy-related proteins—mTOR, phosphorylated mTOR (p-mTOR), autophagy-related protein 7 (Atg7), p62, and microtubule-associated protein light chain 3B-II (LC3B-II)—were significantly elevated in skeletal muscle. However, low-frequency electrical stimulation (LFES) markedly reduced the expression of these autophagy-related proteins, suggesting that ES might reduce MPB by inhibiting autophagy. Kanazashi and Tanaka discovered that two weeks of hindlimb unloading in rats led to a decline in mTORC1 signaling downstream factors p70S6K and S6rp activation and MPS, along with an increase in the autophagy marker LC3-II/I ratio.[66] ES applied to the atrophied muscles successfully activated mTORC1 signaling and decreased the expression of ubiquitinated proteins and the LC3B-II/I ratio, indicating an inhibition of MPB. These findings suggest that ES can suppress MPB when applied to disused skeletal muscles.

ES influences muscle protein synthesis and degradation through the aforementioned pathways, promoting muscle repair and enhancing its function. In addition to the mTOR pathway, ES may also impact several other critical biological signaling pathways,[67,68] such as AMP-activated protein kinase (AMPK) and the pathways of certain growth factors like IGF-1.[69] These pathways collectively regulate muscle growth, repair, and remodeling. By activating these pathways, ES can improve muscle function and increase endurance, further promoting muscle health and recovery. In summary, ES is an effective non-pharmacological therapy that directly stimulates muscles and activates key biochemical pathways, thereby improving muscle function and positively influencing muscle protein synthesis and degradation.

4. The impact of blood flow restriction on muscle tissue

Blood flow restriction (BFR) typically involves applying pressure to a limb (proximal to the trained muscle) using specialized devices such as pneumatic cuffs or elastic bands during exercise. This pressure obstructs venous return and reduces arterial blood flow, causing ischemia in the distal part of the limb.[70] In Japan, this type of training is also known as "kaatsu training." BFR training has been shown to induce muscle adaptations in both clinical populations and athletes and has seen widespread application in exercise training and rehabilitation therapy in recent years.[71–74] However, scholars have proposed various mechanisms to explain the effects of BFR. Most research points to the interplay of multiple potential mechanisms,[75–77] primarily involving metabolic stress responses such as muscle fiber recruitment, activation of protein synthesis signaling pathways, and hormone secretion. Additionally, cell swelling induced by the pressure may contribute to adaptive responses in the muscle (Fig. 3).

4.1. Enhancement of fast-twitch muscle fibers activation and hypertrophy

During BFR, activation of type III and IV afferent nerve fibers and inhibition of α-motor neurons are stimulated due to decreased oxygen and high accumulation of metabolites,[78,79] thereby increasing fiber recruitment to maintain muscle strength(Fig. 3). According to Henneman's size principle of muscle fibers recruitment, slow-twitch fibers are the first to be utilized during exercise. As exercise intensity increases, the recruitment of high-threshold fast-twitch fibers progressively rises.[80] However, when blood flow back to the heart is blocked in the local muscle, oxygen content and phosphocreatine decrease and hydrogen ions increase at the site of pressurization, resulting in a decrease in the pH of the internal environment.[81,82] This hypoxic and acidic condition impedes muscle metabolism and elevates the perceived level of fatigue. Under these conditions, the activation threshold of muscle fibers is reduced, making it easier to activate fast-twitch (FT) muscle fibers even during low-intensity resistance training. The hypoxic conditions caused by vascular occlusion may recruit more motor units,[83,84] further enhancing muscle fibers recruitment. Electromyography (EMG) studies have shown increased recruitment of FT muscle fibers during Kaatsu training.[85,86] Another study on low-intensity blood flow restriction training demonstrated that due to inadequate oxygen delivery, type I muscle fibers experienced early fatigue. Consequently, in a two-week training regimen at 20% of 1 RM, the cross-sectional area of type II muscle fibers increased by 27.6%, while that of type I fibers increased by only 5.9%.[87] In addition, the rapid accumulation of metabolites, such as lactate, results in a mismatch between metabolic demand and supply, which accelerates muscle fiber fatigue and triggers the recruitment of fast-shrinking muscle fibers.[88] Metabolic stress during BFR caused by the hypoxic environment seems to be the main mechanism of enhanced hypertrophy of BFR during low-intensity strength training, as suggested by Suga et al.[89]

In summary, blood flow restriction training (BFRT) subjects muscles to hypoxic and acidic conditions, stimulating adaptive responses in muscle fibers, including enhanced activation and recruitment of fast-twitch (FT) fibers. This is highly beneficial for the growth of strength and muscle mass.

Furthermore, BFR training can increase the cross-sectional area (CSA) of muscle fibers, particularly fast-twitch (FT) fibers, under low-load conditions, as demonstrated by numerous studies. Takarada et al. found that in elderly women, low-intensity occlusion exercise (LIO) at approximately 50%–30% of 1 RM with about 110 mmHg pressure resulted in similar increases in elbow flexor CSA and isokinetic strength as high-to-moderate intensity exercise (HI) at approximately 80%–50% of 1 RM without occlusion, and significantly greater increases than low-intensity exercise (LI) without occlusion.[90] This suggests that resistance exercise with intensities even below 50% of 1 RM, when combined with vascular occlusion, can effectively induce muscle hypertrophy and increase strength. Another study by Takarada et al. also indicated that low-intensity resistance exercise (50% 1 RM) combined with vascular occlusion (200 mmHg) significantly increased isokinetic knee extension torque and quadriceps CSA compared to low-intensity exercise without occlusion or no exercise, thus promoting muscle hypertrophy.[91] Additionally, dynamic endurance of the quadriceps improved following BFR. Sudo et al.'s researth on rats further elucidated that repeated BFR and

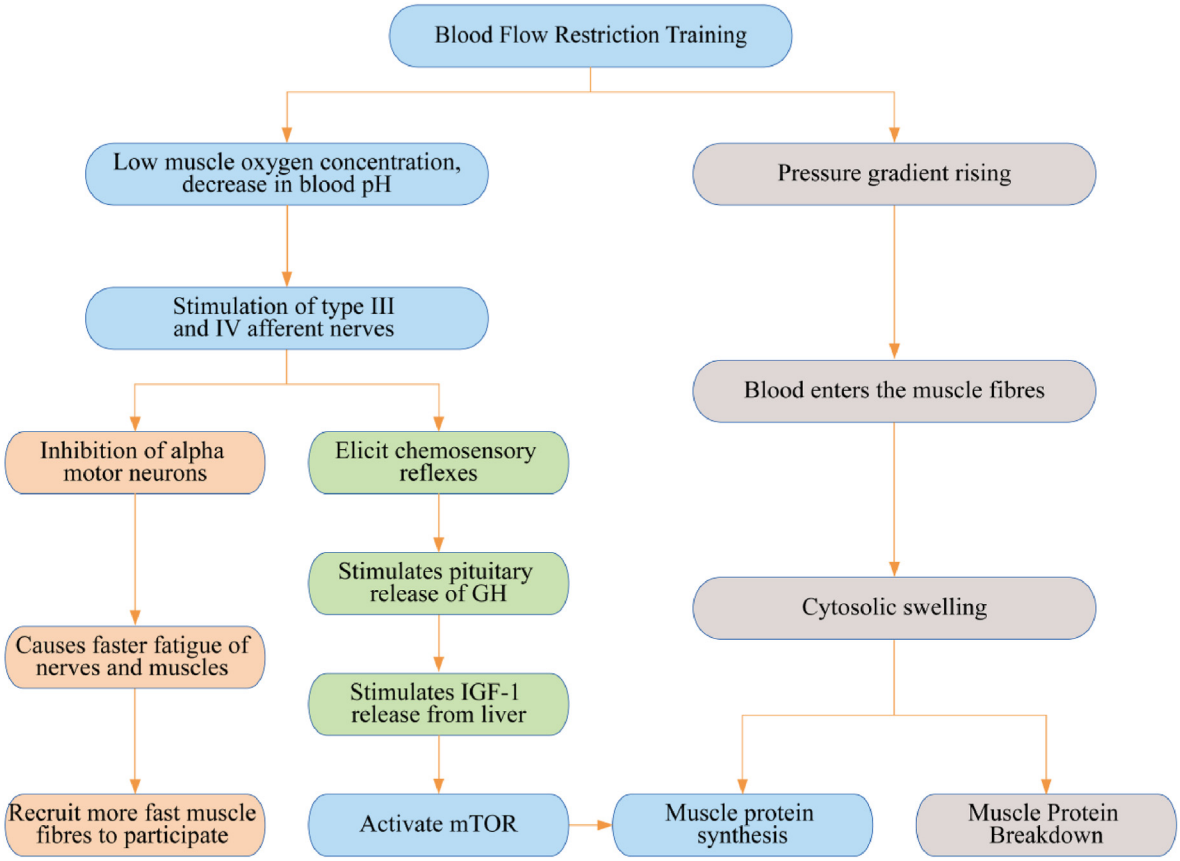

Fig. 3. BFR accelerates the muscle growth process.
Abbreviations: GH, Growth Hormone; IGF-1, Insulin-like growth factor-1; mTOR, mechanistic target of rapamycin.
Blood flow restrictive training creates a low oxygen environment that stimulates afferent nerve fibres and inhibits alpha motor neurons, thereby accelerating the recruitment of fast-acting muscle fibres. The low PH environment increases the activity of growth factors such as IGF-1, which activates the mTOR signalling pathway and stimulates muscle protein synthesis. Blood entry into muscle fibres due to increased pressure causes cell swelling, which also promotes protein synthesis and inhibits protein hydrolysis.

eccentric contractions (ECC) combined with BFR induce muscle fiber hypertrophy at the cellular level.[92] A study on older adults demonstrated that just six weeks of low-load blood flow restriction resistance exercise (BFRRE) resulted in a roughly 20% increase in CSA of both type I and type II fibers, along with improvements in maximal muscle strength and endurance.[93] Libardi et al.'s six-week study on low-load BFR resistance training (LL-BFR) revealed significant increases in CSA of type II and average (type I + type II) muscle fibers,[94] with proportional expansion of both myofibrillar and non-myofibrillar regions. This indicates that LL-BFR, similar to traditional high-load resistance training (HL-RT), can contribute to the hypertrophy of type II fibers and induce skeletal muscle hypertrophy. However, some studies also concluded that low-intensity resistance combined with blood flow restriction training is not as effective as traditional high-intensity resistance training in increasing muscle strength, but it is better than traditional low-load resistance training.[95] In conclusion, low-intensity blood flow restriction training is an effective method of building muscle strength for people of all ages and fitness levels and may be a better choice for those with physical limitations.

The environment created by BFR induces a unique physiological state that effectively activates fast-twitch (FT) muscle fibers, which are particularly important for explosive activities requiring high intensity and short duration. In this way, training conditions at lower intensities may in some ways achieve similar results to high intensity training. Blood flow restriction training enhances the recruitment of fast-contracting muscle fibers compared to load-and-volume matched exercise training, which may contribute to muscle strength gain. Furthermore, blood flow restriction training can improve muscle endurance performance because it forces muscles to work under conditions of limited oxygen supply, thereby enhancing their adaptability to anaerobic exercise. Overall, blood flow restriction training enhances muscle strength, endurance, and overall performance through these mechanisms.

4.2. *Activation of the mTOR pathway in muscle protein synthesis*

BFR can lead to the metabolic accumulation of biomarkers such as whole blood lactate, plasma lactate, and intramuscular lactate,[84,87] creating an acidic environment within the muscle. Additionally, the accumulation of metabolites can affect oxygen delivery to the muscles, as the arterial and venous blood flow is continuously compressed during restricted blood flow exercise, potentially leading to acute hypoxia and a decrease in intramuscular pH levels.[85] This low pH environment, resulting from metabolic accumulation, can stimulate the secretion of growth hormone (GH), which may interact with muscle protein synthesis (Fig. 3). One study found that implementing Kaatsu training resulted in post-exercise GH levels that were ten times higher than those in the control group without blood flow restriction.[85,96] Loenneke et al. also observed that BFR training could increase GH levels to approximately 290.[84] Some research suggests that the primary function of GH is to assist the overall process of skeletal muscle hypertrophy by promoting the release of insulin-like growth factor 1 (IGF-1).[85] Other studies indicate that muscle protein synthesis may require the combined effects of GH and IGF-1 concentrations.[85,97] Takano et al. found that increased IGF-1 activity is a response to low-intensity occlusion training.[98] Additionally, other studies have shown that during blood flow restriction resistance

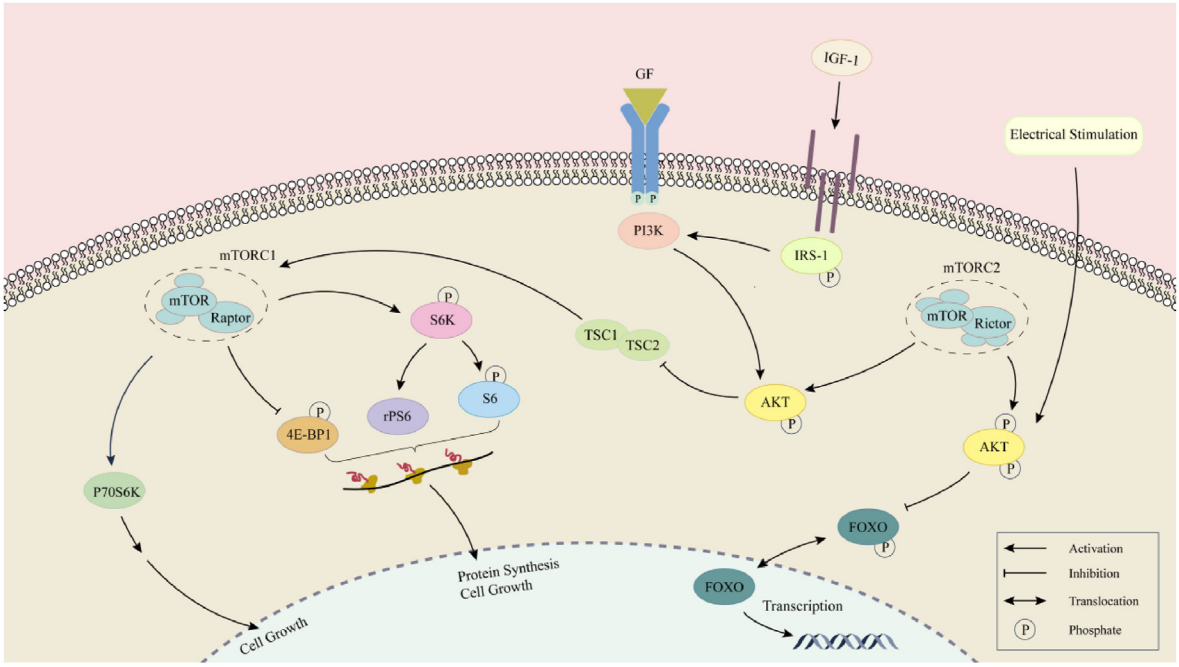

Fig. 4. Schematic representation of muscle protein regulation through the mTOR pathway.
Abbreviations: 4E-BP1, eukaryotic initiation factor 4E binding protein 1; Akt(PKB), protein kinase B; BFR, Blood Flow Restriction; ES, Electrical Stimulation; FT, fast-twitch muscle fibers; GH, Growth Hormone; IGF-1, Insulin-like growth factor-1; mTOR, mechanistic target of rapamycin; mTORC1, mTOR Complex 1; mTORC2, mTOR Complex 2; PI3K, phosphatidylinositol 3-kinase; S6K, ribosomal protein S6 Kinase.
Mechanical tension and metabolic stress induced by blood flow restriction training leads to activation of PI3K, which in turn activates Akt; Akt further directly activates mTORC1 through phosphorylation and inhibition of TSC2. mTORC1 activation marks the initiation of protein synthesis because mTORC1 phosphorylates its downstream effector molecules 4E-BP1 and S6K1. 4E-BP1 phosphorylation by mTORC1 initiates translation initiation; S6K1 activates ribosomal protein S6 after phosphorylation by mTORC1. Phosphorylation of 4E-BP1 by mTORC1 initiates translation initiation; after S6K1 is phosphorylated by mTORC1, S6K1 activates ribosomal protein S6, which increases the translation efficiency of mRNA and promotes protein synthesis. Electrical stimulation also promotes muscle protein synthesis via the AKT/mTOR pathway, and also inhibits the FoxO transcription factor by reducing its nuclear translocation, thereby inhibiting the activity of the ubiquitin-proteasome pathway, which reduces the expression of genes related to protein hydrolysis and inhibits protein catabolism.

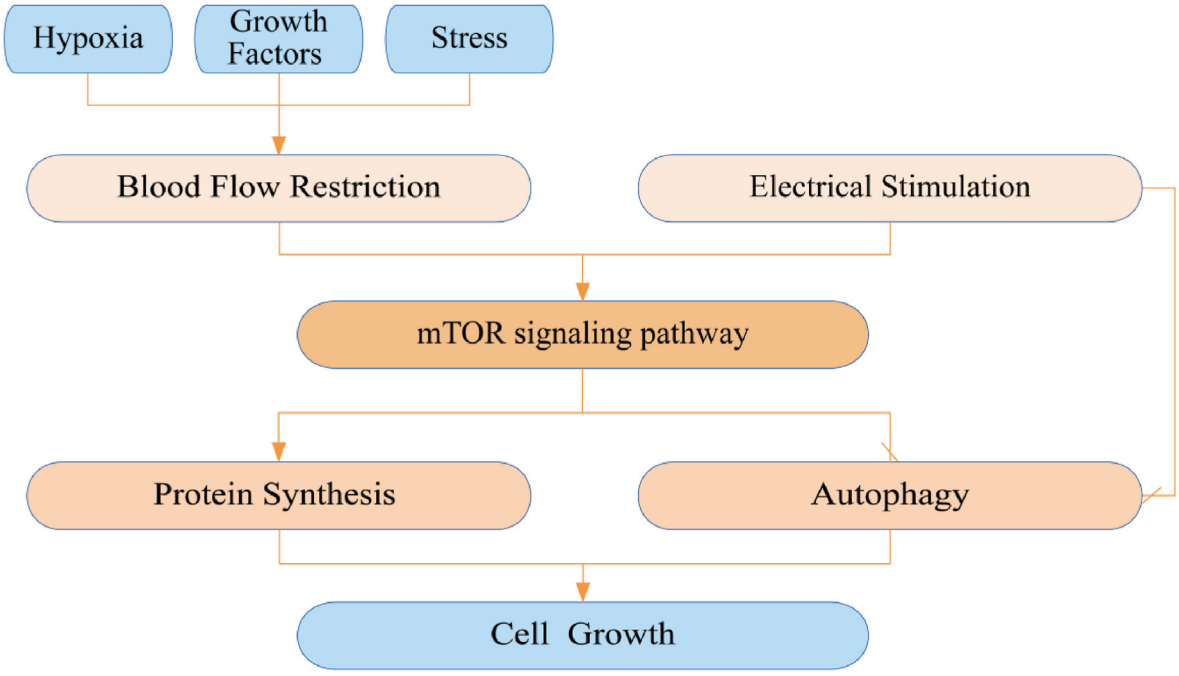

Fig. 5. Schematic diagram of the combined effects of Blood Flow Restriction and Electrical Stimulation.
Blood flow restriction training and electrical stimulation promote muscle protein synthesis and inhibit muscle protein catabolism via the mTOR signalling pathway to promote cell growth and improve muscle strength.

training, circulating levels of IGF-1 increase in correlation with muscle volume growth (Fig. 3).[97,99]

Skeletal muscle growth occurs when the balance between protein synthesis and degradation shifts towards synthesis. Multiple intracellular signaling pathways facilitate muscle growth through these adaptive molecular signals. The IGF-1/PI3K/Akt signaling pathway plays a critical role in regulating muscle mass by stimulating overall protein synthesis and inhibiting protein degradation,[58,100,101] thereby promoting muscle hypertrophy. mTOR is essential in exercise-induced muscle protein synthesis and training-induced hypertrophy.[58,102–104] In skeletal muscle, MPS is achieved by activating the insulin-like growth factor 1-phosphoinositide 3-kinase-Akt/protein kinase B-mammalian target of rapamycin (IGF1-PI3K-Akt/PKB-mTOR) signaling pathway and downregulating the myostatin-Smad 3 pathway, a negative regulator of skeletal muscle growth.[85,105,106] Once Akt stimulates MPS by activating mTOR and its downstream effectors, the mTOR kinase interacts with proteins to form two major complexes[105,106]: mTOR complex 1 (mTORC1), containing raptor, and mTOR complex 2 (mTORC2), containing rictor.[105,106] In the Akt/mTOR pathway, the two primary effectors of mTORC1 that lead to MPS are eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1).[105] Phosphorylation of S6K1 is a critical regulator of exercise-induced muscle protein synthesis (Fig. 4)(See Fig. 5).

Multiple studies have demonstrated that BFR exercise effectively increases the phosphorylation of S6K1, activates mTORC1, and further promotes muscle protein synthesis (MPS) and muscle hypertrophy. Research has shown that low-intensity (20% 1 RM) resistance exercise combined with BFR (200 mmHg) can stimulate the mTOR signaling pathway through its associated downstream effectors, including the phosphorylation of ribosomal S6 kinase 1 (S6K1) and ribosomal protein S6 (rpS6).[96] Drummond et al. investigated acute low-intensity (20% 1 RM) resistance exercise in elderly men and found that the BFR group exhibited a significant increase in the phosphorylation of S6K1 and rpS6, indicating enhanced mTORC1 signaling following BFR exercise.[107] The enhancement of mTORC1 signaling suggests an improvement in translation initiation, which is likely the reason for the increase in MPS induced by BFR. Their recent report indicated that the contraction-induced increase in MPS depends on the activation of mTORC1 in human muscle, supporting their finding that BFR exercise activates mTORC1, playing a crucial role in stimulating MPS and promoting muscle hypertrophy over time.[108] Nakajima et al. provided animal data that also observed increased phosphorylation of p70S6K and ribosomal S6 in skeletal muscle tissue following repeated use of REST + BFR compared to REST alone.[109]

In summary, BFR induces metabolic accumulation, which subsequently activates key signaling pathways and regulates specific protein synthesis effectors. Together, these components form a complex and intricate regulatory network that effectively promotes muscle protein synthesis (MPS) and increases muscle volume.

5. Combined application of electrical stimulation and blood flow restriction

5.1. The combination of blood flow restriction alone with electrical stimulation

Previous research has shown that blood flow restriction (BFR) training can prevent disuse muscle atrophy and reduce muscle weakness caused by chronic unloading.[71,110,111] While BFR can increase metabolic product accumulation within muscles, its effectiveness may be limited without concurrent active muscle contractions. This limitation may prevent full activation of muscle growth-related signaling pathways, such as the mTOR pathway, thereby failing to effectively promote muscle protein synthesis or adequately inhibit protein breakdown. A study measured the effects of BFR during strict bed rest periods on muscle protein synthesis rates, muscle mass, and strength.[112] Results indicate that there was no difference in muscle protein synthesis rates during bed rest between the BFR group and the control group, suggesting that BFR alone without muscle contractions does not regulate daily muscle protein synthesis rates, nor does it maintain muscle mass or strength. Research by Iversen et al. after anterior cruciate ligament reconstruction surgery also indicated that standalone BFR does not reduce postoperative muscle atrophy.[113] Additionally, Nyakayiru et al. found no difference in synthetic metabolic signaling or muscle fiber protein synthesis rates between the rest combined with BFR group and the rest group under resting conditions,[114] suggesting that BFR does not increase muscle fiber protein synthesis rates during rest conditions.

To overcome this limitation, combining electrical stimulation (ES) can be employed. ES induces involuntary muscle contractions, providing an alternative training method when active movement is not feasible.[115] However, ES also has constraints as it requires high-frequency stimulation to evoke muscle contractions, which can lead to discomfort and significant muscle fatigue.[116,117] ES primarily triggers contractions by depolarizing motor axons, recruiting motor units (MUs) synchronously with each stimulation pulse.[118] This necessitates higher ES frequencies to generate sufficiently strong tetanic contractions, forcing motor units to discharge at non-physiologically high rates. Increased metabolic demands on muscle fibers and the reduced excitability of motor axons beneath the stimulating electrode lead to “drop out” of motor units during ES,[119] resulting in elevated fatigue due to the higher discharge rates of motor units during stimulation.[116]

Therefore, combining both may reduce the limitations observed when each is used alone, and some studies have already explored this combination. For instance, Slysz et al. investigated the effects of blood flow restriction (BFR) combined with electrical muscle stimulation (EMS) on skeletal muscle mass and strength during limb disuse.[120] Results indicated that unlike BFR alone, the combination of blood flow restriction with muscle electrical stimulation could mitigate the loss of muscle mass during limb disuse, representing an effective intervention strategy, although it did not conclusively demonstrate strength preservation. Natsume et al. also found that combining neuromuscular electrical stimulation (NMES) with blood flow restriction (BFR) increased leg muscle thickness and maximal knee extension strength after 2 weeks of training in untrained young men,[121] both during isometric and isotonic voluntary contractions. This suggests that low-intensity NMES-BFR induces muscle hypertrophy and strength gains in untrained young male participants. However, these studies did not involve active movement by participants; hence, combining these interventions with active muscle contractions could potentially yield more significant effects.

5.2. The combination of blood flow restricted exercise with electrical stimulation

As previously discussed, electrical stimulation (ES) requires higher frequencies to induce significant muscle contractions, which can cause discomfort and rapid muscle fatigue in patients. Moreover, the metabolic accumulation and improvement in muscle strength from blood flow restriction (BFR) alone are relatively limited without active movement. Therefore, compared to the therapy combining BFR alone with ES, integrating low-intensity exercise with BFR and lower-intensity ES may avoid discomfort from high current intensity and stimulate beneficial muscle growth and strength gains. This combination enhances muscle protein synthesis (MPS) to a certain extent and accelerates the process of muscle growth and recovery (Fig. 4). On one hand, BFR restricts blood flow proximal to the limb, prompting muscles to operate in hypoxic and acidic conditions. This environmental stimulus triggers adaptive responses in muscles, including the recruitment of fast-twitch (FT) fibers and the release of growth factors such as GH and IGF-1. Increased secretion of growth factors activates the mTOR signaling pathway, enhances S6K1 phosphorylation, and directly promotes MPS. On the other hand, ES directly stimulates muscle fibers through electrical currents, inducing muscle contractions. ES activates multiple intracellular

signaling pathways to promote muscle hypertrophy and enhance MPS, thereby contributing to muscle mass maintenance. Firstly, ES activates the Akt signaling pathway, a key mechanism for inducing muscle hypertrophy, which enhances protein synthesis within muscle cells and promotes muscle volume increase. Activation of Akt subsequently indirectly activates the mTOR pathway, upregulating phosphorylation of p70S6K, which accelerates muscle growth. Additionally, electrical stimulation can inhibit the ubiquitin-proteasome pathway in muscles, reducing muscle protein breakdown (MPB) and aiding in maintaining existing muscle mass, particularly crucial in conditions of muscle injury or disease.

In studies such as that by Nyakayiru et al.,[114] combining low-load resistance exercise with blood flow restriction (BFR) increased muscle fiber protein synthesis rates by 10% ± 5% compared to both low-load resistance exercise alone and BFR during rest. Additionally, the combined group exhibited a higher phosphorylation status of 4E-BP1, indicating that BFR can enhance muscle fiber protein synthesis rates following physical activity. Nakajima et al. found that low-intensity electrical stimulation (EXER) combined with BFR enhances ribosomal protein S6 phosphorylation.[122] Yoshikawa et al. found that BFR alone or ES alone did not induce muscle hypertrophy or increase phosphorylation of rpS6 Ser240/244, whereas combined BFR + ES treatment increased muscle mass, fiber cross-sectional area, and phosphorylation of rpS6 Ser240/244 and rpS6 Ser235/236.[123] Furthermore, combined BFR and low-current ES treatment induces muscle hypertrophy by activating ERK1/2 and mTOR protein synthesis signaling pathways. Li et al. demonstrated that combining low-intensity resistance training with blood flow restriction training (BFRT) enhances lower limb muscle strength by promoting muscle hypertrophy and improving muscle activation.[124]

In conclusion, the combination of BFR and ES can synergistically enhance MPS, potentially more effectively than either method alone, thereby promoting muscle growth and recovery. Restricting muscle blood flow while applying electrical stimulation not only improves muscle strength and endurance but also enhances muscle tissue adaptability. This could offer potential benefits in restoring muscle function post-peripheral nerve injury (PNI), accelerating the recovery of muscle strength following PNI.

6. Conclusion

BFR combined with electrical stimulation of ES works synergistically to increase muscle strength and endurance for optimized muscle adaptation. First, ES, as a non-conventional training tool, stimulates the muscle directly by means of electric current, which is capable of inducing contraction of muscle fibers, especially those that are more difficult to activate during conventional training. This stimulation helps to promote a switch in muscle fiber type from fast-contracting to slow-contracting, a switch that enhances muscle endurance and overall performance. Secondly blood flow restriction training creates a hypoxic and acidic environment by restricting blood flow to the limb through the application of external pressure. This environment and metabolic accumulation stimulates FT activation, recruitment and muscle hypertrophy in the muscle, which may enhance muscle cross-sectional area and strength output. When BFR is used in conjunction with ES, the two approaches work together to activate the mTOR signaling pathway, a key intracellular pathway responsible for regulating MPS and muscle growth. In this way, muscle cells are strengthened in terms of MPS, which supports muscle recovery and growth.

Although electrical stimulation combined with blood flow restriction (BFR) training has been studied by researchers for its potential therapeutic efficacy and application value, safety considerations remain paramount. Commentaries analyzing the long-term safety of peripheral nerve electrical stimulation suggest that compared to intraneural electrodes,[125] extraneural electrodes demonstrate superior long-term stability. Adjusting relevant parameters such as frequency and pulse intensity for electrical stimulation is considered a safe intervention method. Regarding the safety of blood flow restriction training, researchers focused on BFR have proposed a series of guidelines.[126] These guidelines emphasize considerations such as vascular responses, venous thrombosis, and muscle damage. While the occurrence of these risks remains contentious, adverse events during training can be minimized through rigorous screening processes, personalized treatment plan adjustments, and preventive measures. Stavres et al. studied the feasibility of blood flow restriction exercise in patients with incomplete spinal cord injury,[127] finding that individuals could safely perform controlled BFR exercise without increasing cardiovascular fatigue or exacerbating pain. This supports the application of BFR in neurological injuries. There are also researchers who have systematically evaluated the safety and effectiveness of blood flow restriction exercise on skeletal muscle size, strength, and functional performance in individuals with neurological disorders, including spinal cord injury, inclusion body myositis, multiple sclerosis, Parkinson's disease, and stroke, and have found that BFR appears to be a potentially safe and effective form of training for individuals with neurological disorders.[128] However, this assessment also had the limitations of limited quality and quantity of studies, small sample sizes, and a general lack of heterogeneity within and between the cohorts of patients examined. Loenneke et al. concluded that the risk of injury or adverse events from blood flow limiting exercise may be consistent with that of conventional exercise when patients are screened for contraindications and when appropriate training methodology, training loads, cuff pressures, and equipment are used.[129] And when performed by appropriately trained and qualified professionals, BFRT can be used safely in appropriate patient populations.[130] In conclusion, BFR training offers an alternative approach to achieving exercise intensity,[131] and current research supports its use in the absence of contraindications. In populations where contraindications do not exist and where traditional progressive resistance training is appropriate, BFR training appears to be a safe and effective approach to therapeutic exercise in the sports medicine setting.

Future research could apply this combined approach to the recovery of muscle strength following peripheral nerve injury (PNI), further exploring the optimal parameters for this combined training. This includes determining the optimal frequency and intensity of electrical stimulation to enhance muscle response and adaptability, and identifying the appropriate pressure levels and duration for blood flow restriction to precisely control training effects. Such optimization aims to reduce the risk of injury associated with high-load training, achieve safe and personalized training protocols, and promote the recovery of muscle strength post-PNI.

CRediT authorship contribution statement

Xiaolei Chu: Writing – original draft. **Jiaojiao Sun:** Writing – original draft. **Jiajia Liang:** Writing – review & editing. **Wenjie Liu:** Writing – review & editing. **Zheng Xing:** Writing – review & editing. **Qi Li:** Writing – review & editing. **Qingwen Li:** Writing – review & editing.

Declaration of competing interest

We confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with

respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

References

1. Padovano WM, Dengler J, Patterson MM, et al. Incidence of nerve injury after extremity trauma in the United States. *Hand*. 2022;17(4):615–623. https://doi.org/10.1177/1558944720963895.
2. Li R, Liu Z, Pan Y, Chen L, Zhang Z, Lu L. Peripheral nerve injuries treatment: a systematic review. *Cell Biochem Biophys*. 2014;68(3):449–454. https://doi.org/10.1007/s12013-013-9742-1.
3. Loenneke JP, Wilson GJ, Wilson JM. A mechanistic approach to blood flow occlusion. *Int J Sports Med*. 2010;31(1):1–4. https://doi.org/10.1055/s-0029-1239499.
4. Brocherie F, Babault N, Cometti G, Maffiuletti N, Chatard JC. Electrostimulation training effects on the physical performance of ice hockey players. *Med Sci Sports Exerc*. 2005;37(3):455–460. https://doi.org/10.1249/01.mss.0000155396.51293.9f.
5. Gregory CM, Bickel CS. Recruitment patterns in human skeletal muscle during electrical stimulation. *Phys Ther*. 2005;85(4):358–364. https://doi.org/10.1093/ptj/85.4.358.
6. Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. *Physiol Rev*. 2011;91(4):1447–1531. https://doi.org/10.1152/physrev.00031.2010.
7. Pette D, Staron RS. Myosin isoforms, muscle fiber types, and transitions. *Microsc Res Tech*. 2000;50(6):500–509. https://doi.org/10.1002/1097-0029(20000915)50:6<500::AID-JEMT7>3.0.CO;2-7.
8. Salanova M, Gelfi C, Moriggi M, et al. Disuse deterioration of human skeletal muscle challenged by resistive exercise superimposed with vibration: evidence from structural and proteomic analysis. *FASEB J*. 2014;28(11):4748–4763. https://doi.org/10.1096/fj.14-252825.
9. Smerdu V, Karsch-Mizrachi I, Campione M, Leinwand L, Schiaffino S. Type IIx myosin heavy chain transcripts are expressed in type IIb fibers of human skeletal muscle. *Am J Physiol*. 1994;267(6 Pt 1):C1723–C1728. https://doi.org/10.1152/ajpcell.1994.267.6.C1723.
10. Weiss S, Rossi R, Pellegrino MA, Bottinelli R, Geeves MA. Differing ADP release rates from myosin heavy chain isoforms define the shortening velocity of skeletal muscle fibers. *J Biol Chem*. 2001;276(49):45902–45908. https://doi.org/10.1074/jbc.M107434200.
11. Matsakas A, Patel K. Skeletal muscle fibre plasticity in response to selected environmental and physiological stimuli. *Histol Histopathol*. 2009;24(5):611–629. https://doi.org/10.14670/HH-24.611.
12. Ohlendieck K. Proteomic profiling of fast-to-slow muscle transitions during aging. *Front Physiol*. 2011;2:105. https://doi.org/10.3389/fphys.2011.00105.
13. Jackson HE, Ingham PW. Control of muscle fibre-type diversity during embryonic development: the zebrafish paradigm. *Mech Dev*. 2013;130(9-10):447–457. https://doi.org/10.1016/j.mod.2013.06.001.
14. Hoppeler H. Molecular networks in skeletal muscle plasticity. *J Exp Biol*. 2016;219(Pt 2):205–213. https://doi.org/10.1242/jeb.128207.
15. Pette D. The adaptive potential of skeletal muscle fibers. *Can J Appl Physiol*. 2002;27(4):423–448. https://doi.org/10.1139/h02-023.
16. Moriggi M, Vasso M, Fania C, et al. Long term bed rest with and without vibration exercise countermeasures: effects on human muscle protein dysregulation. *Proteomics*. 2010;10(21):3756–3774. https://doi.org/10.1002/pmic.200900817.
17. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat Rev Drug Discov*. 2015;14(1):58–74. https://doi.org/10.1038/nrd4467.
18. Chargé SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev*. 2004;84(1):209–238. https://doi.org/10.1152/physrev.00019.2003.
19. Bodine SC, Latres E, Baumhueter S, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science*. 2001;294(5547):1704–1708. https://doi.org/10.1126/science.1065874.
20. Bonaldo P, Sandri M. Cellular and molecular mechanisms of muscle atrophy. *Dis Model Mech*. 2013;6(1):25–39. https://doi.org/10.1242/dmm.010389.
21. Marimuthu K, Murton AJ, Greenhaff PL. Mechanisms regulating muscle mass during disuse atrophy and rehabilitation in humans. *J Appl Physiol (1985)*. 2011;110(2):555–560. https://doi.org/10.1152/japplphysiol.00962.2010.
22. Jokl P, Konstadt S. The effect of limb immobilization on muscle function and protein composition. *Clin Orthop Relat Res*. 1983;174:222–229. https://doi.org/10.1097/00003086-198304000-00031.
23. Booth FW. Effect of limb immobilization on skeletal muscle. *J Appl Physiol Respir Environ Exerc Physiol*. 1982;52(5):1113–1118. https://doi.org/10.1152/jappl.1982.52.5.1113.
24. Liu X, Yuan H, Niu Y, Niu W, Fu L. The role of AMPK/mTOR/S6K1 signaling axis in mediating the physiological process of exercise-induced insulin sensitization in skeletal muscle of C57BL/6 mice. *Biochim Biophys Acta*. 2012;1822(11):1716–1726. https://doi.org/10.1016/j.bbadis.2012.07.008.
25. Levitt DE, Luk HY, Vingren JL. Alcohol, resistance exercise, and mTOR pathway signaling: an evidence-based narrative review. *Biomolecules*. 2022;13(1):2. https://doi.org/10.3390/biom13010002.
26. D'Hulst G, Palmer AS, Masschelein E, Bar-Nur O, De Bock K. Voluntary resistance running as a model to induce mTOR activation in mouse skeletal muscle. *Front Physiol*. 2019;10:1271. https://doi.org/10.3389/fphys.2019.01271.
27. Yin L, Lu L, Lin X, Wang X. Crucial role of androgen receptor in resistance and endurance trainings-induced muscle hypertrophy through IGF-1/IGF-1R- PI3K/Akt-mTOR pathway. *Nutr Metab*. 2020;17:26. https://doi.org/10.1186/s12986-020-00446-y.
28. Bai I, Keyser C, Zhang Z, et al. Epigenetic regulation of autophagy in neuroinflammation and synaptic plasticity. *Front Immunol*. 2024;15:1322842. https://doi.org/10.3389/fimmu.2024.1322842.
29. Kataoka R, Hammert WB, Yamada Y, et al. The plateau in muscle growth with resistance training: an exploration of possible mechanisms. *Sports Med*. 2024;54(1):31–48. https://doi.org/10.1007/s40279-023-01932-y.
30. Ogasawara R, Jensen TE, Goodman CA, Hornberger TA. Resistance exercise-induced hypertrophy: a potential role for rapamycin-insensitive mTOR. *Exerc Sport Sci Rev*. 2019;47(3):188–194. https://doi.org/10.1249/JES.0000000000000189.
31. Masui K, Tanaka K, Akhavan D, et al. mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. *Cell Metabol*. 2013;18(5):726–739. https://doi.org/10.1016/j.cmet.2013.09.013.
32. Van Riggelen J, Yetil A, Felsher DW. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer*. 2010;10(4):301–309. https://doi.org/10.1038/nrc2819.
33. Rion N, Castets P, Lin S, Enderle L, Reinhard JR, Rüegg MA. mTORC2 affects the maintenance of the muscle stem cell pool. *Skeletal Muscle*. 2019;9(1):30. https://doi.org/10.1186/s13395-019-0217-y.
34. Kirschbaum BJ, Schneider S, Izumo S, Mahdavi V, Nadal-Ginard B, Pette D. Rapid and reversible changes in myosin heavy chain expression in response to increased neuromuscular activity of rat fast-twitch muscle. *FEBS Lett*. 1990;268(1):75–78. https://doi.org/10.1016/0014-5793(90)80976-p.
35. Wehrle U, Düsterhöft S, Pette D. Effects of chronic electrical stimulation on myosin heavy chain expression in satellite cell cultures derived from rat muscles of different fiber-type composition. *Differentiation*. 1994;58(1):37–46. https://doi.org/10.1046/j.1432-0436.1994.5810037.x.
36. Windisch A, Gundersen K, Szabolcs MJ, Gruber H, Lømo T. Fast to slow transformation of denervated and electrically stimulated rat muscle. *J Physiol*. 1998;510(Pt 2):623–632. https://doi.org/10.1111/j.1469-7793.1998.623bk.x.
37. Mabuchi K, Szvetko D, Pintér K, Sréter FA. Type IIB to IIA fiber transformation in intermittently stimulated rabbit muscles. *Am J Physiol*. 1982;242(5):C373–C381. https://doi.org/10.1152/ajpcell.1982.242.5.C373.
38. Leeuw T, Pette D. Coordinate changes in the expression of troponin subunit and myosin heavy-chain isoforms during fast-to-slow transition of low-frequency-stimulated rabbit muscle. *Eur J Biochem*. 1993;213(3):1039–1046. https://doi.org/10.1111/j.1432-1033.1993.tb17851.x.
39. Brownson C, Little P, Jarvis JC, Salmons S. Reciprocal changes in myosin isoform mRNAs of rabbit skeletal muscle in response to the initiation and cessation of chronic electrical stimulation. *Muscle Nerve*. 1992;15(6):694–700. https://doi.org/10.1002/mus.880150611.
40. Andersen JL, Mohr T, Biering-Sørensen F, Galbo H, Kjaer M. Myosin heavy chain isoform transformation in single fibres from m. vastus lateralis in spinal cord injured individuals: effects of long-term functional electrical stimulation (FES). *Pflügers Archiv*. 1996;431(4):513–518. https://doi.org/10.1007/BF02191897.
41. Nuhr M, Crevenna R, Gohlsch B, et al. Functional and biochemical properties of chronically stimulated human skeletal muscle. *Eur J Appl Physiol*. 2003;89(2):202–208. https://doi.org/10.1007/s00421-003-0792-8.
42. Gondin J, Brocca L, Bellinzona E, et al. Neuromuscular electrical stimulation training induces atypical adaptations of the human skeletal muscle phenotype: a functional and proteomic analysis. *J Appl Physiol (1985)*. 2011;110(2):433–450. https://doi.org/10.1152/japplphysiol.00914.2010.
43. Termin A, Staron RS, Pette D. Changes in myosin heavy chain isoforms during chronic low-frequency stimulation of rat fast hindlimb muscles. A single-fiber study. *Eur J Biochem*. 1989;186(3):749–754. https://doi.org/10.1111/j.1432-1033.1989.tb15269.x.
44. Aigner S, Pette D. Fast-to-slow transition in myosin heavy chain expression of rabbit muscle fibres induced by chronic low-frequency stimulation. *Symp Soc Exp Biol*. 1992;46:311–317.
45. Kirschbaum BJ, Heilig A, Härtner KT, Pette D. Electrostimulation-induced fast-to-slow transitions of myosin light and heavy chains in rabbit fast-twitch muscle at the mRNA level. *FEBS Lett*. 1989;243(2):123–126. https://doi.org/10.1016/0014-5793(89)80112-7.
46. Nuhr MJ, Pette D, Berger R, et al. Beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure. *Eur Heart J*. 2004;25(2):136–143. https://doi.org/10.1016/j.ehj.2003.09.027.
47. Abdellaoui A, Préfaut C, Gouzi F, et al. Skeletal muscle effects of electrostimulation after COPD exacerbation: a pilot study. *Eur Respir J*. 2011;38(4):781–788. https://doi.org/10.1183/09031936.00167110.
48. Toth MJ, Voigt TB, Tourville TW, et al. Effect of neuromuscular electrical stimulation on skeletal muscle size and function in patients with breast cancer receiving chemotherapy. *J Appl Physiol (1985)*. 2020;128(6):1654–1665. https://doi.org/10.1152/japplphysiol.00203.2020.
49. Toth MJ, Tourville TW, Voigt TB, et al. Utility of neuromuscular electrical stimulation to preserve quadriceps muscle fiber size and contractility after anterior cruciate ligament injuries and reconstruction: a randomized, sham-controlled, blinded trial. *Am J Sports Med*. 2020;48(10):2429–2437. https://doi.org/10.1177/0363546520933622.
50. Fan Y, Dickman KG, Zong WX. Akt and c-Myc differentially activate cellular metabolic programs and prime cells to bioenergetic inhibition. *J Biol Chem*. 2010;285(10):7324–7333. https://doi.org/10.1074/jbc.M109.035584.
51. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell*. 2007;129(7):1261–1274. https://doi.org/10.1016/j.cell.2007.06.009.

52. Robey RB, Hay N. Is Akt the "Warburg kinase"?-Akt-energy metabolism interactions and oncogenesis. *Semin Cancer Biol*. 2009;19(1):25–31. https://doi.org/10.1016/j.semcancer.2008.11.010.
53. Shiojima I, Walsh K. Role of Akt signaling in vascular homeostasis and angiogenesis. *Circ Res*. 2002;90(12):1243–1250. https://doi.org/10.1161/01.res.0000022200.71892.9f.
54. An WL, Cowburn RF, Li L, et al. Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. *Am J Pathol*. 2003;163(2):591–607. https://doi.org/10.1016/S0002-9440(10)63687-5.
55. Isotani S, Hara K, Tokunaga C, Inoue H, Avruch J, Yonezawa K. Immunopurified mammalian target of rapamycin phosphorylates and activates p70 S6 kinase alpha in vitro. *J Biol Chem*. 1999;274(48):34493–34498. https://doi.org/10.1074/jbc.274.48.34493.
56. Sasai N, Agata N, Inoue-Miyazu M, et al. Involvement of PI3K/Akt/TOR pathway in stretch-induced hypertrophy of myotubes. *Muscle Nerve*. 2010;41(1):100–106. https://doi.org/10.1002/mus.21473.
57. Navé BT, Ouwens M, Withers DJ, Alessi DR, Shepherd PR. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem J*. 1999;344(Pt 2):427–431. Pt 2.
58. Bodine SC, Stitt TN, Gonzalez M, et al. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol*. 2001;3(11):1014–1019. https://doi.org/10.1038/ncb1101-1014.
59. Sugiura T, Abe N, Nagano M, et al. Changes in PKB/Akt and calcineurin signaling during recovery in atrophied soleus muscle induced by unloading. *Am J Physiol Regul Integr Comp Physiol*. 2005;288(5):R1273–R1278. https://doi.org/10.1152/ajpregu.00688.2004.
60. Ohno Y, Fujiya H, Goto A, et al. Microcurrent electrical nerve stimulation facilitates regrowth of mouse soleus muscle. *Int J Med Sci*. 2013;10(10):1286–1294. https://doi.org/10.7150/ijms.5985.
61. Dirks ML, Hansen D, Van Assche A, Dendale P, Van Loon LJ. Neuromuscular electrical stimulation prevents muscle wasting in critically ill comatose patients. *Clin Sci (Lond)*. 2015;128(6):357–365. https://doi.org/10.1042/CS20140447.
62. Wall BT, Dirks ML, Verdijk LB, et al. Neuromuscular electrical stimulation increases muscle protein synthesis in elderly type 2 diabetic men. *Am J Physiol Endocrinol Metab*. 2012;303(5):E614–E623. https://doi.org/10.1152/ajpendo.00138.2012.
63. Mettler JA, Bennett SM, Doucet BM, Magee DM. Neuromuscular electrical stimulation and anabolic signaling in patients with stroke. *J Stroke Cerebrovasc Dis*. 2017;26(12):2954–2963. https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.07.019.
64. Khodabukus A, Madden L, Prabhu NK, et al. Electrical stimulation increases hypertrophy and metabolic flux in tissue-engineered human skeletal muscle. *Biomaterials*. 2019;198:259–269. https://doi.org/10.1016/j.biomaterials.2018.08.058.
65. Liu AY, Zhang QB, Zhu HL, et al. Low-frequency electrical stimulation alleviates immobilization-evoked disuse muscle atrophy by repressing autophagy in skeletal muscle of rabbits. *BMC Muscoskel Disord*. 2022;23(1):398. https://doi.org/10.1186/s12891-022-05350-5.
66. Kanazashi M, Tanaka M. Acute effect of electrical stimulation on muscle protein synthesis and break-down in the soleus muscle of hindlimb unloaded rats. *Biomed Res*. 2023;44(5):209–218. https://doi.org/10.2220/biomedres.44.209.
67. Hutber CA, Hardie DG, Winder WW. Electrical stimulation inactivates muscle acetyl-CoA carboxylase and increases AMP-activated protein kinase. *Am J Physiol*. 1997;272(2 Pt 1):E262–E266. https://doi.org/10.1152/ajpendo.1997.272.2.E262.
68. Lee K, Ochi E, Song H, Nakazato K. Activation of AMP-activated protein kinase induce expression of FoxO1, FoxO3a, and myostatin after exercise-induced muscle damage. *Biochem Biophys Res Commun*. 2015;466(3):289–294. https://doi.org/10.1016/j.bbrc.2015.08.126.
69. Bayol S, Brownson C, Loughna PT. Electrical stimulation modulates IGF binding protein transcript levels in C2C12 myotubes. *Cell Biochem Funct*. 2005;23(5):361–365. https://doi.org/10.1002/cbf.1118.
70. Scott BR, Loenneke JP, Slattery KM, Dascombe BJ. Exercise with blood flow restriction: an updated evidence-based approach for enhanced muscular development. *Sports Med*. 2015;45(3):313–325. https://doi.org/10.1007/s40279-014-0288-1.
71. Takarada Y, Takazawa H, Ishii N. Applications of vascular occlusion diminish disuse atrophy of knee extensor muscles. *Med Sci Sports Exerc*. 2000;32(12):2035–2039. https://doi.org/10.1097/00005768-200012000-00011.
72. Ohta H, Kurosawa H, Ikeda H, Iwase Y, Satou N, Nakamura S. Low-load resistance muscular training with moderate restriction of blood flow after anterior cruciate ligament reconstruction. *Acta Orthop Scand*. 2003;74(1):62–68. https://doi.org/10.1080/00016470310013680.
73. Takarada Y, Nakamura Y, Aruga S, Onda T, Miyazaki S, Ishii N. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J Appl Physiol (1985)*. 2000;88(1):61–65. https://doi.org/10.1152/jappl.2000.88.1.61.
74. Cook CJ, Kilduff LP, Beaven CM. Improving strength and power in trained athletes with 3 weeks of occlusion training. *Int J Sports Physiol Perform*. 2014;9(1):166–172. https://doi.org/10.1123/ijspp.2013-0018.
75. Yuan J, Wu L, Xue Z, Xu G, Wu Y. Application and progress of blood flow restriction training in improving muscle mass and strength in the elderly. *Front Physiol*. 2023;14:1155314. https://doi.org/10.3389/fphys.2023.1155314.
76. Loenneke JP, Fahs CA, Rossow LM, Abe T, Bemben MG. The anabolic benefits of venous blood flow restriction training may be induced by muscle cell swelling. *Med Hypotheses*. 2012;78(1):151–154. https://doi.org/10.1016/j.mehy.2011.10.014.
77. Schoenfeld BJ. Potential mechanisms for a role of metabolic stress in hypertrophic adaptations to resistance training. *Sports Med*. 2013;43(3):179–194. https://doi.org/10.1007/s40279-013-0017-1.
78. Loenneke JP, Fahs CA, Wilson JM, Bemben MG. Blood flow restriction: the metabolite/volume threshold theory. *Med Hypotheses*. 2011;77(5):748–752. https://doi.org/10.1016/j.mehy.2011.07.029.
79. Yasuda T, Abe T, Brechue WF, et al. Venous blood gas and metabolite response to low-intensity muscle contractions with external limb compression. *Metabolism*. 2010;59(10):1510–1519. https://doi.org/10.1016/j.metabol.2010.01.016.
80. Henneman E, Somjen G, Carpenter DO. Functional significance of cell size Iin spinal motoneurons. *J Neurophysiol*. 1965;28:560–580. https://doi.org/10.1152/jn.1965.28.3.560.
81. Yanagisawa O, Sanomura M. Effects of low-load resistance exercise with blood flow restriction on high-energy phosphate metabolism and oxygenation level in skeletal muscle. *Interv Med Appl Sci*. 2017;9(2):67–75. https://doi.org/10.1556/1646.9.2017.2.16.
82. Suga T, Okita K, Morita N, et al. Intramuscular metabolism during low-intensity resistance exercise with blood flow restriction. *J Appl Physiol (1985)*. 2009;106(4):1119–1124. https://doi.org/10.1152/japplphysiol.90368.2008.
83. Moritani T, Sherman WM, Shibata M, Matsumoto T, Shinohara M. Oxygen availability and motor unit activity in humans. *Eur J Appl Physiol Occup Physiol*. 1992;64(6):552–556. https://doi.org/10.1007/BF00843767.
84. Loenneke JP, Abe T, Wilson JM, Ugrinowitsch C, Bemben MG. Blood flow restriction: how does it work? *Front Physiol*. 2012;3:392. https://doi.org/10.3389/fphys.2012.00392.
85. Schoenfeld BJ. Potential mechanisms for a role of metabolic stress in hypertrophic adaptations to resistance training. *Sports Med*. 2013;43(3):179–194. https://doi.org/10.1007/s40279-013-0017-1.
86. Takarada Y, Nakamura Y, Aruga S, Onda T, Miyazaki S, Ishii N. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J Appl Physiol (1985)*. 2000;88(1):61–65. https://doi.org/10.1152/jappl.2000.88.1.61.
87. Abe T, Kearns CF, Sato Y. Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. *J Appl Physiol (1985)*. 2006;100(5):1460–1466. https://doi.org/10.1152/japplphysiol.01267.2005.
88. Pearson SJ, Hussain SR. A review on the mechanisms of blood-flow restriction resistance training-induced muscle hypertrophy. *Sports Med*. 2015;45(2):187–200. https://doi.org/10.1007/s40279-014-0264-9.
89. Suga T, Okita K, Morita N, et al. Intramuscular metabolism during low-intensity resistance exercise with blood flow restriction. *J Appl Physiol (1985)*. 2009;106(4):1119–1124. https://doi.org/10.1152/japplphysiol.90368.2008.
90. Takarada Y, Takazawa H, Sato Y, Takebayashi S, Tanaka Y, Ishii N. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. *J Appl Physiol (1985)*. 2000;88(6):2097–2106. https://doi.org/10.1152/jappl.2000.88.6.2097.
91. Takarada Y, Sato Y, Ishii N. Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. *Eur J Appl Physiol*. 2002;86(4):308–314. https://doi.org/10.1007/s00421-001-0561-5.
92. Sudo M, Ando S, Kano Y. Repeated blood flow restriction induces muscle fiber hypertrophy. *Muscle Nerve*. 2017;55(2):274–276. https://doi.org/10.1002/mus.25415.
93. Wang J, Mogensen AG, Thybo F, et al. Low-load blood flow-restricted resistance exercise produces fiber type-independent hypertrophy and improves muscle functional capacity in older individuals. *J Appl Physiol (1985)*. 2023;134(4):1047–1062. https://doi.org/10.1152/japplphysiol.00789.2022.
94. Libardi CA, Godwin JS, Reece TM, Ugrinowitsch C, Herda TJ, Roberts MD. Effects of low-load resistance training with blood flow restriction on muscle fiber myofibrillar and extracellular area. *Front Physiol*. 2024;15:1368646. https://doi.org/10.3389/fphys.2024.1368646.
95. Lixandrão ME, Ugrinowitsch C, Berton R, et al. Magnitude of muscle strength and mass adaptations between high-load resistance training versus low-load resistance training associated with blood-flow restriction: a systematic review and meta-analysis. *Sports Med*. 2018;48(2):361–378. https://doi.org/10.1007/s40279-017-0795-y.
96. Fujita S, Abe T, Drummond MJ, et al. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. *J Appl Physiol (1985)*. 2007;103(3):903–910. https://doi.org/10.1152/japplphysiol.00195.2007.
97. Pierce JR, Clark BC, Ploutz-Snyder LL, Kanaley JA. Growth hormone and muscle function responses to skeletal muscle ischemia. *J Appl Physiol (1985)*. 2006;101(6):1588–1595. https://doi.org/10.1152/japplphysiol.00585.2006.
98. Takano H, Morita T, Iida H, et al. Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow. *Eur J Appl Physiol*. 2005;95(1):65–73. https://doi.org/10.1007/s00421-005-1389-1.
99. Abe T, Kearns CF, Sato Y. Muscle size and strength are increased following walk training with restricted venous blood flow from the leg muscle, Kaatsu-walk training. *J Appl Physiol (1985)*. 2006;100(5):1460–1466. https://doi.org/10.1152/japplphysiol.01267.2005.
100. Wernbom M, Apro W, Paulsen G, Nilsen TS, Blomstrand E, Raastad T. Acute low-load resistance exercise with and without blood flow restriction increased protein signalling and number of satellite cells in human skeletal muscle. *Eur J Appl Physiol*. 2013;113(12):2953–2965. https://doi.org/10.1007/s00421-013-2733-5.
101. Rommel C, Bodine SC, Clarke BA, et al. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat Cell Biol*. 2001;3(11):1009–1013. https://doi.org/10.1038/ncb1101-1009.

102. Baar K, Esser K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol*. 1999;276(1):C120–C127. https://doi.org/10.1152/ajpcell.1999.276.1.C120.
103. O'Neil TK, Duffy LR, Frey JW, Hornberger TA. The role of phosphoinositide 3-kinase and phosphatidic acid in the regulation of mammalian target of rapamycin following eccentric contractions. *J Physiol*. 2009;587(Pt 14):3691–3701. https://doi.org/10.1113/jphysiol.2009.173609.
104. Reynolds TH 4th, Bodine SC, Lawrence Jr JC. Control of Ser2448 phosphorylation in the mammalian target of rapamycin by insulin and skeletal muscle load. *J Biol Chem*. 2002;277(20):17657–17662. https://doi.org/10.1074/jbc.M201142200.
105. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS J*. 2013;280(17):4294–4314. https://doi.org/10.1111/febs.12253.
106. Ge Y, Chen J. Mammalian target of rapamycin (mTOR) signaling network in skeletal myogenesis. *J Biol Chem*. 2012;287(52):43928–43935. https://doi.org/10.1074/jbc.R112.406942.
107. Fry CS, Glynn EL, Drummond MJ, et al. Blood flow restriction exercise stimulates mTORC1 signaling and muscle protein synthesis in older men. *J Appl Physiol (1985)*. 2010;108(5):1199–1209. https://doi.org/10.1152/japplphysiol.01266.2009.
108. Drummond MJ, Fry CS, Glynn EL, et al. Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *J Physiol*. 2009;587(Pt 7):1535–1546. https://doi.org/10.1113/jphysiol.2008.163816.
109. Nakajima T, Yasuda T, Koide S, et al. Repetitive restriction of muscle blood flow enhances mTOR signaling pathways in a rat model. *Heart Ves*. 2016;31(10):1685–1695. https://doi.org/10.1007/s00380-016-0801-6.
110. Kubota A, Sakuraba K, Sawaki K, Sumide T, Tamura Y. Prevention of disuse muscular weakness by restriction of blood flow. *Med Sci Sports Exerc*. 2008;40(3):529–534. https://doi.org/10.1249/MSS.0b013e31815ddac6.
111. Kubota A, Sakuraba K, Koh S, Ogura Y, Tamura Y. Blood flow restriction by low compressive force prevents disuse muscular weakness. *J Sci Med Sport*. 2011;14(2):95–99. https://doi.org/10.1016/j.jsams.2010.08.007.
112. Fuchs CJ, Hermans WJH, Nyakayiru J, et al. Daily blood flow restriction does not preserve muscle mass and strength during 2 weeks of bed rest. *J Physiol*. 2024. https://doi.org/10.1113/JP286065. Published online February 27.
113. Iversen E, Røstad V, Larmo A. Intermittent blood flow restriction does not reduce atrophy following anterior cruciate ligament reconstruction. *J Sport Health Sci*. 2016;5(1):115–118. https://doi.org/10.1016/j.jshs.2014.12.005.
114. Nyakayiru J, Fuchs CJ, Trommelen J, et al. Blood flow restriction only increases myofibrillar protein synthesis with exercise. *Med Sci Sports Exerc*. 2019;51(6):1137–1145. https://doi.org/10.1249/MSS.0000000000001899.
115. Doucet BM, Lam A, Griffin L. Neuromuscular electrical stimulation for skeletal muscle function. *Yale J Biol Med*. 2012;85(2):201–215. https://PMID:2273704.
116. Luu MJ, Jones KE, Collins DF. Decreased excitability of motor axons contributes substantially to contraction fatigability during neuromuscular electrical stimulation. *Appl Physiol Nutr Metabol*. 2021;46(4):346–355. https://doi.org/10.1139/apnm-2020-0366.
117. Neyroud D, Dodd D, Gondin J, Maffiuletti NA, Kayser B, Place N. Wide-pulse-high-frequency neuromuscular stimulation of triceps surae induces greater muscle fatigue compared with conventional stimulation. *J Appl Physiol (1985)*. 2014;116(10):1281–1289. https://doi.org/10.1152/japplphysiol.01015.2013.
118. Bergquist AJ, Clair JM, Collins DF. Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae. *J Appl Physiol (1985)*. 2011;110(3):627–637. https://doi.org/10.1152/japplphysiol.01103.2010.
119. Vanderthommen M, Duchateau J. Electrical stimulation as a modality to improve performance of the neuromuscular system. *Exerc Sport Sci Rev*. 2007;35(4):180–185. https://doi.org/10.1097/jes.0b013e318156e785.
120. Slysz JT, Boston M, King R, Pignanelli C, Power GA, Burr JF. Blood flow restriction combined with electrical stimulation attenuates thigh muscle disuse atrophy. *Med Sci Sports Exerc*. 2021;53(5):1033–1040. https://doi.org/10.1249/MSS.0000000000002544.
121. Natsume T, Ozaki H, Saito AI, Abe T, Naito H. Effects of electrostimulation with blood flow restriction on muscle size and strength. *Med Sci Sports Exerc*. 2015;47(12):2621–2627. https://doi.org/10.1249/MSS.0000000000000722.
122. Nakajima T, Koide S, Yasuda T, et al. Muscle hypertrophy following blood flow-restricted, low-force isometric electrical stimulation in rat tibialis anterior: role for muscle hypoxia. *J Appl Physiol (1985)*. 2018;125(1):134–145. https://doi.org/10.1152/japplphysiol.00972.2017.
123. Yoshikawa M, Morifuji T, Matsumoto T, Maeshige N, Tanaka M, Fujino H. Effects of combined treatment with blood flow restriction and low-current electrical stimulation on muscle hypertrophy in rats. *J Appl Physiol (1985)*. 2019;127(5):1288–1296. https://doi.org/10.1152/japplphysiol.00070.2019.
124. Li N, Yang J, Liao Y. The effect of blood flow restriction training combined with electrical muscle stimulation on neuromuscular adaptation: a randomized controlled trial. *Front Physiol*. 2023;14:1182249. https://doi.org/10.3389/fphys.2023.1182249.
125. Günter C, Delbeke J, Ortiz-Catalan M. Safety of long-term electrical peripheral nerve stimulation: review of the state of the art. *J NeuroEng Rehabil*. 2019;16(1):13. https://doi.org/10.1186/s12984-018-0474-8.
126. Patterson SD, Hughes L, Warmington S, et al. Blood flow restriction exercise: considerations of methodology, application, and safety. *Front Physiol*. 2019;10:533. https://doi.org/10.3389/fphys.2019.00533.
127. Stavres J, Singer TJ, Brochetti A, Kilbane MJ, Brose SW, McDaniel J. The feasibility of blood flow restriction exercise in patients with incomplete spinal cord injury. *Pharm Manag PM R*. 2018;10(12):1368–1379. https://doi.org/10.1016/j.pmrj.2018.05.013.
128. Jønsson AB, Krogh S, Laursen HS, Aagaard P, Kasch H, Nielsen JF. Safety and efficacy of blood flow restriction exercise in individuals with neurological disorders: a systematic review. *Scand J Med Sci Sports*. 2024;34(1):e14561. https://doi.org/10.1111/sms.14561.
129. Loenneke JP, Wilson JM, Wilson GJ, Pujol TJ, Bemben MG. Potential safety issues with blood flow restriction training. *Scand J Med Sci Sports*. 2011;21(4):510–518. https://doi.org/10.1111/j.1600-0838.2010.01290.x.
130. Anderson KD, Rask DMG, Bates TJ, Nuelle JAV. Overall safety and risks associated with blood flow restriction therapy: a literature review. *Mil Med*. 2022;187(9-10):1059–1064. https://doi.org/10.1093/milmed/usac055.
131. Lorenz DS, Bailey L, Wilk KE, et al. Blood flow restriction training. *J Athl Train*. 2021;56(9):937–944. https://doi.org/10.4085/418-20.