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STRENGTH TRAINING WITH VASCULAR OCCLUSION: A REVIEW OF POSSIBLE ADAPTIVE MECHANISMS

review article
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ABSTRACT
Strength training with blood flow restriction, or KAATSU training, has been shown to be as effective as conventional strength training to promote muscular strength and hypertrophy. Several mechanisms have been suggested as hypotheses to explain the adaptations arising from this training method. Among these is metabolic stress, which exerts important physiological effects and may influence the training adaptations in question. In addition, hypoxia produced by the technique may change the neural recruitment pattern. Growth hormone (GH) concentrations increase as a result of practicing this method, which can trigger an increase in plasmatic and, perhaps, muscular insulin-like growth factor-1 (IGF-1) concentrations. The increase in concentrations of these factors can play a leading role in responses to KAATSU training. Among the effects of the GH/IGF-1 axis in muscle cells is the increase in the signalling pathway activity of the mammalian target of rapamycin (mTOR), which has been associated with increased protein synthesis. On the other hand, the decrease in the activity of the myostatin pathway, which has an antagonistic effect to mTOR, has been demonstrated after training with occlusion. Other factors, such as increases in the expression of heat shock proteins, may play an important role in adaptations to exercise. Nitric oxide synthase could increase nitric oxide concentration, which in turn has an effect on satellite cells and blood flow. However, despite the results obtained, the transfer to other situations (e.g. speed sports) is not yet clear.
Key words: KAATSU training, hypertrophy, hypoxia, neural recruitment, mTOR, myostatin

Introduction

Training with vascular occlusion (TVO), or KAATSU training, has been widely studied in recent years [1–5]. Research has shown that this method promotes increases in muscular strength and hypertrophy in a manner similar to conventional strength training (CT) [3, 6]. However, many questions, especially with regard to aspects of safety and adaptive mechanisms, still need more information to be fully understood.

The purpose of the article is to provide a descriptive and critical review on the possible adaptive mechanisms to TVO and seek an understanding of its actual workings. To ensure the transparency and reproducibility of the literature search and illustrate the search strategy elements, the principles of a systematic review were adopted. Therefore, searches were conducted in the following databases: Institute for Scientific Information (ISI) Web of Knowledge, Scopus, PubMed, and Google Scholar. For the process of identifying articles, the following keywords were used: *vascular occlusion training* and *KAATSU training*, each associated with the descriptors: *adaptive mechanisms*; *muscle oxygenation*; *metabolic stress*; *neuromuscular responses*; *hormonal responses*; *signalling pathways*. The following inclusion criteria were adopted: 1) empirical scientific articles; 2) studies carried out with human beings and/or animals; 3) full text online; 4) papers containing data considered relevant to the understanding of the adaptive mechanisms of TVO. The present study reports the principal topics studied in the field of adaptive mechanisms of TVO and the main effects arising from this training strategy. The results obtained are discussed and the major limitations are described. The search outcome was the total of 27 articles. After a detailed analysis of each paper, a categorization system was implemented, contemplating seven themes of analysis:

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1) muscle oxygenation and metabolic stress; 2) muscle fibre type recruitment; 3) growth hormone (GH); 4) mammalian target of rapamycin (mTOR) signalling pathway; 5) myostatin; 6) heat shock proteins; 7) nitric oxide synthase (NOS).

Muscle oxygenation and metabolic stress

During TVO, the muscles of the occluded region are affected by a decrease in oxygenation during exercise [1, 7]. As aerobic metabolism is dependent on the presence of oxygen, it may be supposed that anaerobic metabolism would have a greater participation in the energy supply during TVO. Low levels of hypoxia promote an increase in metabolic enzymatic activity toward greater aerobic balance while severe hypoxia changes the metabolism to greater anaerobic potential [8]. Thus, at the level of hypoxia in which the O_2 availability is not saturated for the cytochrome oxidase, metabolic adaptations to compensate and maintain the adenosine triphosphate (ATP) flow through the aerobic metabolism are performed, while more extreme levels of hypoxia result in an additional need of glycolysis to support ATP production owing to the decreased ability of mitochondrial cytochromes to reduce O_2 in H_2O because of limitations depending on O_2 availability [8].

Since the ATP hydrolysis rate is higher than the production rate of ATP via mitochondrial respiration, the H^+ proton accumulation increases intramuscular acidosis. The lactate dehydrogenase reaction serves as a pathway for proton consumption in the conversion of pyruvate to lactate [9]. The lactate leaves the muscle cell through specific transporters called monocarboxylate transporters (MCTs) together with an H^+ proton [10]. The lactate formed is transported through the bloodstream to the liver, where it is converted to glucose, or to other tissues, where it can be used as an energy source, such as the heart or other muscle groups [11]. Thus, blood lactate concentration can be used as an indirect measure of the metabolic demand [9].

It has already been amply demonstrated that the response of plasmatic lactate in humans [1, 4, 12–14] is increased after a TVO session, especially after the removal of the occlusion pressure. Furthermore, chronic venous flow restriction promotes increased muscle lactate concentration in rats 14 days after occlusive surgery [15]. The large accumulation of intramuscular protons can inhibit the release of Ca^{2+} from the sarcoplasmic reticulum, preventing the excitation-contraction of myofilaments, reducing the development of pressure as manifested in peripheral muscle fatigue [9]. Thus, as the concentration of H^+ protons increases within the cell, its removal becomes necessary owing to its toxicity to the intracellular environment, thereby it is removed together with lactate through the MCTs in order to prevent the early occurrence of fatigue. However, because of intra- and extra-muscular buffers, the increase in lactate concentration and decrease in pH do not correlate linearly [11].

During TVO, venous return is hindered and/or inhibited, depending on the pressure exerted by the *cuff*, as the venous system is a low pressure system compared with the arterial system. Thus, as the lactate concentration and pH between the interior of the fibre and blood plasma become close, these metabolites tend to accumulate in muscle cells [9, 16]. Some evidence has shown that blood lactate reaches its maximum level after the release of the occlusion pressure [1, 17]. Therefore, TVO causes an increase in lactate concentration and a decrease in pH, both within the muscle fibres during training and in the bloodstream after exercise and release of blood flow (Figure 1).

Muscle fibre type recruitment

The recruitment of the types of fibres in general follows the size, or Henneman, principle, where the motor units (MUs) with lower thresholds are recruited first and the larger thresholds later, as the exercise intensity increases [18]. However, in TVO, the higher threshold MUs which innervate the fast twitch fibres can be re-

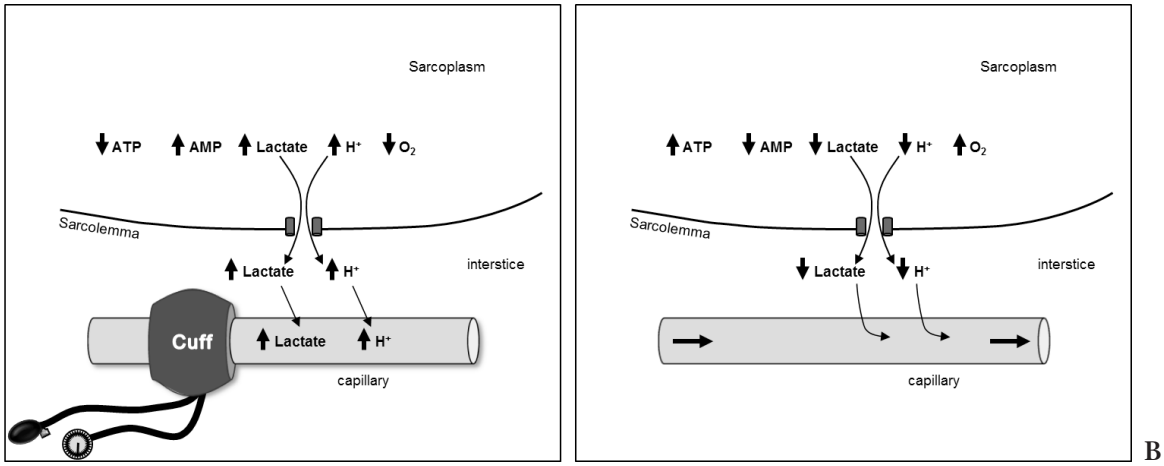

Figure 1. Metabolic effects during (A) and after (B) exercise with vascular occlusion. In A, there is no clearance of lactate and H^+ because of absence of venous circulation; in B, the restoration of blood flow permits the metabolite clearance

cruited even with low-intensity exercise (Figure 2) [9, 16]. By being more susceptible to an increase in cross-section area, the activation of the fast-twitch fibres is crucial to achieve a significant hypertrophic response [9]. In fact, in a study of Moritani et al. [19], the researchers observed an increase in peak amplitude and frequency of firings of MUs consistent with increased recruitment of high-threshold MUs in individuals who underwent manual isometric holds with occlusion when compared with a situation without occlusion. This fact could be attributed to muscle fatigue that can alter the activation of MUs and order of recruitment of muscle fibres according to the Henneman principle [9].

Despite evidence concerning fatigue as an active variable in TVO to alter the pattern of recruitment, Wernbom et al. [20] found no difference in the pattern of electromyographic (EMG) activity, either in concentric or eccentric contractions of individuals who underwent knee extensions to failure with and without occlusion. In addition, long periods of TVO can reduce EMG activity owing to a probable increase in sensory *feedback* or increased perceived exertion during occluded exercise, which inhibits motor activity [9].

Several studies have shown an increase in EMG activity during a TVO session [5, 21–23]. In a research of Yasuda et al. [22], a higher EMG activity was found both in the triceps and pectoralis major muscles in the bench press exercise in individuals who performed the exercise with occlusion in the upper limbs, compared with the control group. Furthermore, in order to establish the relative exercise intensity, the authors observed that in the third set of 15 repetitions, the integrated electromyogram (iEMG) average for the percentage of 1 repetition-maximum (1RM) was around 60–70% for the brachial triceps and pectoral major in the occluded group, while in the control situation this variable was around 50% for both muscles. Similar results (data not shown) were found in another study from the same group, where acting synergistically, the iEMG in the pectoral and deltoid muscles was higher for exercise with occlusion, as a likely mechanism to compensate for the deficit in the power development in the triceps during the bench press with occlusion [24].

Chronically, it has been demonstrated that TVO promotes an increase in surface EMG activity measured by the root mean square (RMS) for the maximum voluntary contraction (MVC) in 3 and 30 seconds, and stimulates a substantial decrease (11–14%) in the percentage of RMS relative to the MVC test in 3 seconds in a resistance test with a load of 20% of 1RM [25]. These data led the authors to suggest that TVO could promote an increase in the number of MUs recruited and/or in the firing frequency, as noted in the behaviour of the RMS in MVCs of 3 and 30 seconds, as well as lower recruitment or frequency of MU firings required to perform the same relative task. However, a meta-analysis by Loenneke et al. [26] proposes the hypothesis that TVO reverses the adaptations arising from CT, hypertrophy being the factor responsible for increases in strength in the early stages, followed by neural adjustments.

In summary, TVO promotes an increase in the EMG activity of active muscles suffering the direct effects of blood restriction. In addition, occlusion may not have an effect on the recruitment of MUs when the exercise is performed until concentric failure. Chronically, this method of training may promote an increase in the recruitment and/or frequency of firings of MUs, as well as a higher efficiency in the ability to generate muscular strength, although such effects are still controversial.

Growth hormone

An overload of the anaerobic system imposed by TVO promotes an increase in the intracellular concentration of some metabolites, which in turn stimulates the afferent pathways III and IV, and may cause alterations in GH secretion [27]. Among these metabolites, highlighted are: H^+ protons, lactate, and adenosine monophosphate (AMP) [9, 16]. During TVO, these metabolites are held back within the muscle, with an increase in lactate and H^+ protons in the bloodstream after release of blood flow following removal of the occlusion pressure [9, 16].

Kraemer and Ratamess [28] pointed at the high correlation between blood lactate and serum concentrations of GH, and maintained that the accumulation of H^+ could be the primary factor in the release of this hormone. In fact, Kraemer et al. [29] demonstrated that strength training protocols which produced higher levels of blood lactate were also those that showed higher increases in GH concentration. Wahl et al. [30] observed that individuals supplemented with bicarbonate and subjected to 4 maximum efforts of 30 seconds for 5 minutes of rest on a cycle ergometer demonstrated a lower GH concentration 10 minutes after the effort as com-

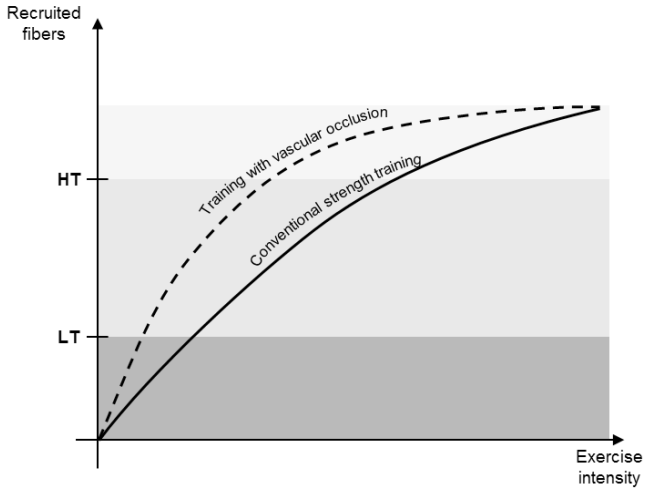

Figure 2. Recruitment of muscle fibres in training with vascular occlusion and conventional strength training. HT – high threshold, LT – low threshold

pared with the placebo condition, since the pH of the subjects supplemented with bicarbonate presented a smaller reduction than in the placebo group. These data corroborate the thesis that there is a strong relationship between metabolic acidosis and GH secretion.

Despite the role of GH as an effective anabolic agent being questionable, it appears to stimulate collagen synthesis, which could have a positive effect on the protection against ruptures, allowing heavier training with short recovery periods [31]. Furthermore, GH could exert an indirect anabolic effect by stimulating the synthesis of hepatic insulin-like growth factor-1 (IGF-1) [32], which corresponds to the largest part of this circulating growth factor [33]. Although muscle cells are also able to synthesize IGF-1 in an autocrine and paracrine manner [33–35] in response to mechanical stimulation, evidence has suggested that the expression of messenger RNA (mRNA) and/or protein expression of the factor itself is suppressed in GH deficiency [33]; nevertheless, this does not prevent a hypertrophic response to mechanical stress [34]. However, there appears to be no additional effect of administering recombinant human GH (rhGH) in subjects with normal secretion of this hormone on IGF-1 expression [33].

Several studies using TVO have demonstrated that a session promotes an increase in GH concentration [1, 4, 12, 14, 21, 27, 36–39]. In many of these, an increase in blood lactate concentration was observed [1, 4, 12, 14, 21], which could partly explain the increase in GH secretion as explained above. Inagaki et al. [17] demonstrated that after an electrostimulation session, the blood lactate level only rose after blood restoration, while in the experiment without occlusion blood lactate was already high immediately after the stimulus. Furthermore, the values of GH increased only on the occasion of blood flow restriction. These data support the hypothesis of stimulation of afferent pathways III and IV for increasing GH secretion, possibly explaining in part the observed increase in several studies.

Some studies have failed to observe an increase in the concentration of IGF-1 after a TVO session [1, 12]. However, a study of Abe et al. [40] showed an increase in serum levels of IGF-1 at the end of a TVO protocol in subjects who exercised twice daily for two weeks, six times per week, at the intensity of 20% 1RM in squat and knee flexion exercises. However, Yasuda et al. [24], using a similar methodology in the bench press exercise, did not observe alterations in this growth factor. Additionally, an increase in IGF-1 expression was not demonstrated in the plantar muscles of mice subjected to chronic occlusion induced surgically [15].

Some points could explain such discrepancies. Firstly, the studies analysing IGF-1 response after TVO in an acute manner were performed over a short period, extending from immediately after training to 3 hours after stimulation. This period of time may be insufficient to note significant alterations in IGF-1 plasma levels, since, as mentioned previously, circulating IGF-1 is predominantly hepatic, being synthesized in response to stimulation by GH, a process that takes 8–30 hours [32]. Thus, owing to this length of time required for the synthesis of these growth factors in response to stimulation by GH, any occurrence of IGF-1 in the bloodstream during or immediately after exercise could suggest the rupture of fibres that have already been contained [28, 32].

Secondly, the expression of IGF-1 on the part of muscle fibres is dependent on the mechanical stimulus applied. Thus, low-intensity exercise may not impose a sufficiently intense mechanical load to stimulate the expression of these factors. As for the discrepancies between the works of Abe et al. [40] and Yasuda et al. [24], these may reflect the differences between the periods of time for the collection of blood samples after training; in the first study, samples were collected on the day following the end of the training protocol after an overnight fasting period (< 24 hours), while in the second, samples were taken 2 days after the end of the protocol after an overnight fasting period (> 48 hours). Thus, this difference in the time period for obtaining blood samples after the end of the exercise protocol may have affected the concentration of this factor through several mechanisms, such as degradation rate, clearance, or interaction with receptors [28]. Furthermore, the larger volume of exercises in the study of Abe et al. [40] could have led to higher production of lactate and H^+ protons, which could impact the levels of GH and consequently IGF-1.

In summary, TVO promotes increases in GH secretion due to metabolic acidosis caused by hypoxia in muscle fibres arising from blood flow restriction or inhibition by occlusion pressure. This increase in GH secretion could stimulate the expression and release of IGF-1 by the liver and thus be an important factor related to the muscle adaptations observed in TVO (Figure 3).

mTOR signalling pathway

The target of rapamycin (TOR), or mTOR, is an evolutionarily conserved protein kinase and an important agent in the control of cell growth [41]. The mTOR interacts with several proteins to form two distinct complexes: mTOR complex 1 (mTORC1) and 2 (mTORC2) [42]. The mTORC1 controls many important processes, including the synthesis of lipids and proteins, as well as autophagy, while mTORC2 regulates metabolism, cell survival, apoptosis, growth, proliferation, ionic transport, and cellular organization through the control of members of the AGC sub-family of kinases (protein kinase B [or Akt], serum- and glucocorticoid-induced protein kinase 1 [SGK1], and protein kinase C-α [PKC-α]) [42].

The mTOR pathway has been identified as important in muscle growth following CT [43–46], and the activation of S6 kinase 1 (S6K1), also p70S6K, has been suggested as being involved in adaptive growth responses to chronic CT [44]. As TVO promotes similar responses

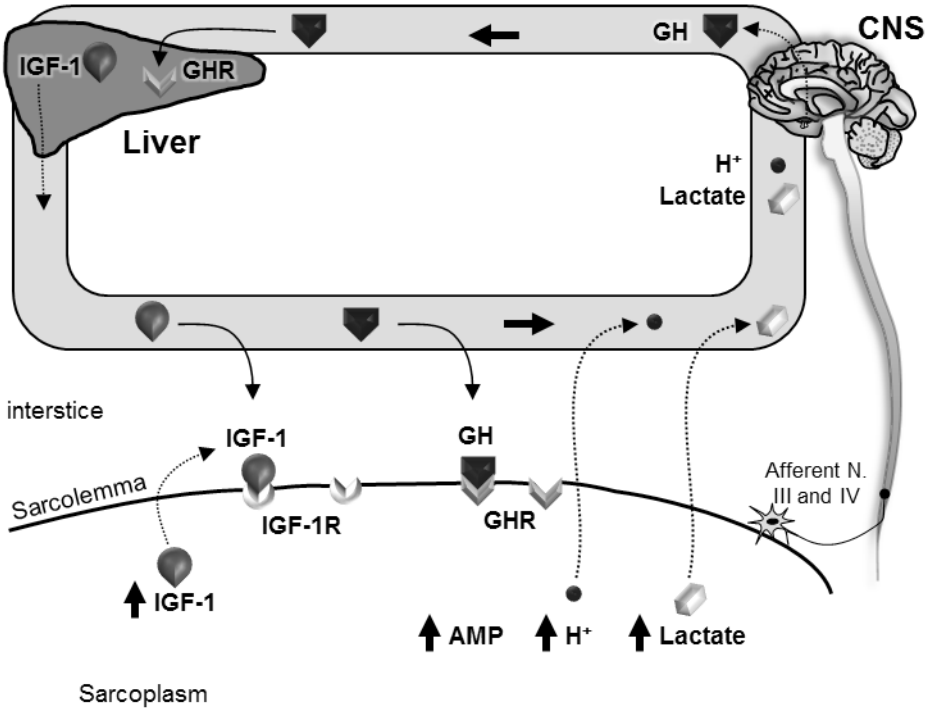

Figure 3. Illustration of the possible mechanisms acting in the release of GH and IGF-1. Lactate and H^+ in blood or in the cell can stimulate the GH release from hypothalamus-hypophysis axis, thus promoting the expression and release of IGF-1 from liver, which in its turn acts over the cells, binding to its receptor (IGF-1R). Note that GH receptors are present on hepatic and other cells. IGF-1 can also be produced in an autocrine or paracrine manner. AMP – adenosine monophosphate, CNS – central nervous system, GH – growth hormone, GHR – growth hormone receptor, IGF-1 – insulin-like growth factor-1, IGF-1R – insulin-like growth factor-1 receptor

to CT [3, 6], it could be thought that this pathway also has great participation in the adaptations from this type of training.

In fact, one of the hypotheses to explain the observed effects on muscle hypertrophy and, in part, muscle strength following TVO is the increase in protein synthesis through the mTOR pathway [16]. Some studies have analysed the activity of mTOR [1, 2, 12, 13] or the expression of mRNA [47, 48] for this specific protein and its targets downstream and upstream.

TVO has been shown to increase the S6K1 phosphorylation at the Thr389 site, both in young individuals [2, 12, 13] and elderly males [1], activating it. However, there appears not to be a change in mRNA expression of this protein after a TVO session [47]. Despite the use of the mRNA expression of specific genes as an indicator of changes in the expression of certain proteins, this may be insufficient as an indicator of protein expression [49, 50]. Furthermore, the activation of many proteins is affected by numerous factors, from metabolic and hormonal issues to complex interactions with other proteins.

S6K1 is a downstream target of mTOR and the latter can activate the former both by phosphorylation of the Thr389 site and by phosphorylation and subsequent inactivation of a phosphatase protein which inactivates S6K1 [51]. In addition, S6K1 is inhibited by the immunosuppressor rapamycin (mTOR inhibitor), which strongly supports its relationship with mTOR [51]. Thus, a high level of phosphorylation of S6K1 has been accompanied by a high rate of muscle protein synthesis after TVO [1, 2, 12, 13].

Gundermann et al. [13] verified that TVO did not alter the state of phosphorylation of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) on the Thr37/46 site at either of the moments (1 hour and 3 hours after the stimulus), a fact confirmed by Fry et al. [1]. mTOR promotes the phosphorylation of 4E-BP1, which negatively acts on the control of protein synthesis, on the bases Thr37/46, Thr70, and Ser65, decreasing the activity of this protein [52]. However, a tumour suppressor protein p53 has been shown to control the dephosphorylation of 4E-BP1 and the inhibition of translation through mTORC1-dependent effects [53]. Furthermore, p53, which plays an important role in metabolism and mitochondrial biogenesis, may present increased activity during physical exercise through its phosphorylation by adenosine monophosphate-activated protein kinase (AMPK) and/or p38 (a mitogen-activated protein kinase [MAPK]), and this effect is triggered by increased mechanical *stress*, Ca^{2+}, reactive oxygen species (ROS), the AMP/ATP ratio, and a decrease in glycogen [54]. Thus, this information could explain why the studies of Gundermann et al. [13] and Fry et al. [1]

found no increase in the phosphorylation of 4E-BP1, even with increased mTOR activity.

Immediately, 1, and 3 hours after a TVO session, Fry et al. [1] found no differences in the protein expression of hypoxia inducible factor-1α (HIF-1α) or regulated in development and DNA damage response 1 (REDD1). However, Drummond et al. [47] found increases in the mRNA expression of HIF-1α and a decrease in mRNA of REDD1. Hypoxia tends to increase the activity of mTOR inhibitory factors such as REDD1/2, as hypoxia is a signal inhibitor of mTOR [42, 55]. In fact, hypoxia causes a rapid and reversible hypophosphorylation of mTOR, 4E-BP1, S6K1 [56]. Another factor that increases during hypoxia is HIF-1α [57, 58]. REDD1 can be induced in an HIF-dependent fashion, although it has been observed that REDD1 is induced in response to hypoxia in embryonic stem HIF-1$\alpha^{-/-}$ cells, although to a lesser extent [59]. However, the decrease in the activity of mTOR in response to hypoxia may be independent of HIF-1α [56], suggesting that the induction of REDD1 by hypoxia may also be mediated by other members of the HIF-α family (such as HIF-2α) or perhaps by an HIF-independent mechanism [59].

The absence of alterations in protein expression of either of the above two factors at any of the moments indicates that hypoxia during a TVO does not occur to any great extent [1]. Methodological differences such as in vivo versus cell culture, oxygenation level (74.5 + 4.8% of oxygen saturation versus 1.5% O_2), and exposure time to hypoxia (4–5 minutes of occlusion versus 30 minutes incubated) may have affected the different responses between the studies of Fry et al. [1] and Arsham et al. [56], respectively. Furthermore, mTORC1 increases glycolytic flux by activating the transcription and translation of HIF-1α, a positive regulator of many glycolytic genes [42], which may explain the data of Drummond et al. [47], possibly related to the adaptations to TVO and not to hypoxia itself.

Regarding the activity of mTOR, the study by Fujita et al. [12] revealed no alterations in the phosphorylation of this at the Ser2448 site 3 hours after a TVO session but Fry et al. [1] found an increase in the phosphorylated/total ratio in the same site of mTOR in elderly men. Gundermann et al. [13] observed that the phosphorylation of mTORC1 at the Ser2448 site was increased as compared with baseline values and higher than that in the control group after TVO. Another study by Gundermann et al. [2], with a view to verifying whether the signalling of mTORC1 is required for the stimulation of muscle protein synthesis after a TVO session, proved that phosphorylation of mTOR at the Ser2448 site was increased 3 hours after a training session in young adults, and differences for the group treated with rapamycin were found 6 and 24 hours after the stimulus. Furthermore, Drummond et al. [47] did not demonstrate acute alterations in mRNA expression for mTOR in 6 young male individuals. To our knowledge, only one study has so far assessed the expression of transcription factors in a chronic manner, among which is mTOR [48]. In this case study, a 65-year-old male patient with inclusion body myositis presented a slight increase in the mRNA expression of mTOR after 12 weeks of TVO [48].

The increase or no alteration in the phosphorylation of mTOR in the Ser2448 base is not the only form of activation of this kinase, since other phosphorylation sites have been identified, such as Ser2481, Thr2446, and Ser1261, which has been found to be the only one that regulates the function of mTOR [52]. The phosphorylation of the Ser2448 base of mTOR has been suggested to be mediated by Akt [52, 60], although recent studies have recognized S6K1 as the kinase of mTOR of Ser2448 [52, 61, 62]. The Ser2481 base is an autophosphorylation site of mTOR, its phosphorylation being higher in the mTORC2 complex than in the mTORC1; the site Ser1261, which remains with the identity of its kinase still unknown, promotes increased catalytic activity of mTORC1, phosphorylation of substrates mediated by mTORC1, and cell growth [52]. Therefore, the activity of mTOR is influenced by phosphorylation of several sites.

Fujita et al. [12] observed a tendency for an increase in the phosphorylation of Akt on the Ser473 site 3 hours after TVO, while Fry et al. [1] found an increase in the phosphorylated/total ratio of Akt on the Thr308 site 3 hours after a similar protocol, although in elderly individuals. However, immediately after the TVO session and before the release of blood flow, no alteration on Akt Thr308 was detected [1]. Another study showed that there was an increase in phosphorylation of Akt at Thr308 1 hour after a TVO session [13]. Although Akt is not a direct mTOR kinase, it can influence the activity of the latter by regulating the activity of tuberous sclerosis 1/2 (TSC1/2), phosphorylating the TSC2 in at least two sites (Thr1462 and Ser939), and inactivating it [55]. Akt is phosphorylated on Thr308 by phosphoinositide-dependent kinase 1 (PDK1), a kinase belonging to the phosphatidyl inositol 3-kinase (PI3K) pathway, while the phosphorylation of Ser473 is by mTORC2 [41, 52]. Thus, an increase in the concentration of local and systemic growth factors which activate the PI3K pathway could impact on the activity of Akt and, consequently, mTOR.

Other signalling pathways, such as the pathways of MAPK, which are activated during exercise [63], are likely to play an important role in the increase in the protein synthesis process, particularly extracellular signal-regulated kinase 1/2 (ERK1/2) and its downstream targets such as MAP kinase-interacting kinase 1 (Mnk1) [1, 2, 13]. In addition, ERK1/2 can affect the activity of mTOR through phosphorylation and subsequent inactivation of TSC2 at the Ser664 site [64]. Therefore, TVO promotes increased muscle protein synthesis, which appears to be, in part, due to the increase in the activity of the mTOR signalling pathway. Figure 4 summarizes that point.

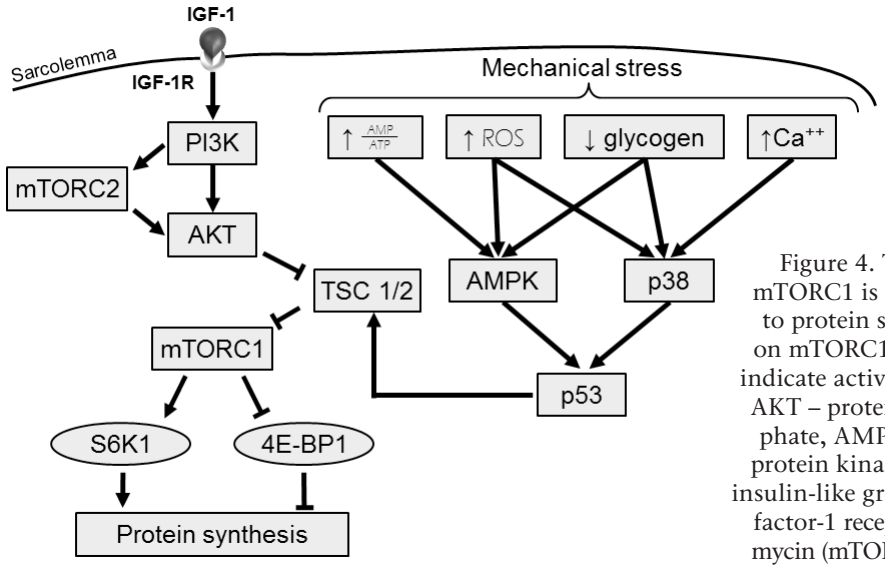

Figure 4. The mTOR signalling pathway in TVO. mTORC1 is activated by IGF-1/PI3K pathway, leading to protein synthesis. p53 can also exert some effects on mTORC1, mainly during an exercise bout. Arrows indicate activation, and blocked lines denote inhibition. AKT – protein kinase B, AMP – adenosine monophosphate, AMPK – adenosine monophosphate-activated protein kinase, ATP – adenosine triphosphate, IGF-1 – insulin-like growth factor-1, IGF-1R – insulin-like growth factor-1 receptor, mTORC1 – mammalian target of rapamycin (mTOR) complex 1, mTORC2 – mTOR complex 2, p38 – a mitogen-activated protein kinase, p53 – a tumour suppressor protein, PI3K – phosphatidyl inositol 3-kinase, ROS – reactive oxygen species, S6K1 – S6 kinase 1, TSC 1/2 – tuberous sclerosis 1/2, 4E-BP1 – eukaryotic translation initiation factor 4E-binding protein 1

Myostatin

Myostatin, also known as growth and differentiation factor-8 (GDF-8), is a family member of tumour growth factor β (TGF-β) [65]. Myostatin is a negative regulator of muscle growth and mutations in myostatin gene result in exaggerated development of muscle in mice, cattle, and humans [16]. It has already been documented that acute CT decreases myostatin mRNA expression and, in the long term, CT reduces myostatin levels in humans and rats [66].

TVO could affect the regulation of myostatin [9, 16]. In fact, in rats subjected to chronic restriction of venous blood flow, a decrease in myostatin in the plantaris muscle [15] and a decrease in mRNA expression in humans after TVO have already been documented [6, 47]. Laurentino et al. [6], analysing mRNA expression of several genes involved in myostatin pathways, found a decrease for myostatin and an increase for follistatin, follistatin-like 3, growth and differentiation factor-associated serum protein 1 (GASP-1), and MAD-related protein-7 (SMAD-7) after 8 weeks of TVO.

Myostatin exerts an inhibitory effect on muscle growth owing to its connection with type IIA (ActRIIA) or IIB activin receptor (ActRIIB), the affinity for the latter being greater than for the former, and promoting activation by the phosphorylation of SMAD-2 and SMAD-3, followed by oligomerization with SMAD-4, which form a complex that is translocated into the nucleus, where it regulates transcription of genes such as MyoD (a transcriptional factor involved in developing skeletal muscle and repairing damaged skeletal muscle) [65, 66].

Specific inhibitors can prevent binding of myostatin to its receptors, among which are: follistatin; follistatin-like 3, which has 30% homology with follistatin and may also bind to circulating myostatin; and GASP-1, which can also bind to myostatin propeptide, binding myostatin in the blood stream, leaving it in a physiologically inactive form, and regulating its activity [65]. Some SMADs (SMAD-6 and SMAD-7) act as regulators against the signs of TGF-βs, functioning as a negative *feedback* [65, 66]. Therefore, highly expressed and/or activated inhibitory factors together with the decreased concentration and/or activity of myostatin can lead to an increase in muscle development and may play a role in the similar adaptive responses between CT and TVO [6].

Drummond et al. [47] observed a decrease in myostatin mRNA expression and an increase in MyoD and *muscle RING finger-1* (MuRF-1) after exercise sessions of low-intensity resistance with and without occlusion. As stated above, the signal from myostatin through the SMADs regulates transcription of the gene of MyoD, decreasing its transcriptional activity, although myostatin also inhibits the expression of Pax3, which is possibly an upstream target of MyoD [65]. Thus, a decrease in the concentration and/or activity of myostatin could allow an increase in the transcription of the gene of MyoD, possibly promoting an increase in its concentration and an elevation in activity.

Myostatin also affects the level of phosphorylation of Akt, which plays a significant role in different metabolic processes in the cell, affecting the activity of other metabolic pathways, such as: Akt/FoxO, Akt/GSK-3β, Akt/mTOR/S6K1 [65]. When Akt is hypophosphorylated, phosphorylation of *forkhead box O* (FoxO) is decreased, allowing it to enter into the cell nucleus and stimulate the transcription of two factors that act in protein degradation, atrogin-1 (also MAFbx) and MuRF-1 [65]. In addition, the increase in nuclear factor-kappa B

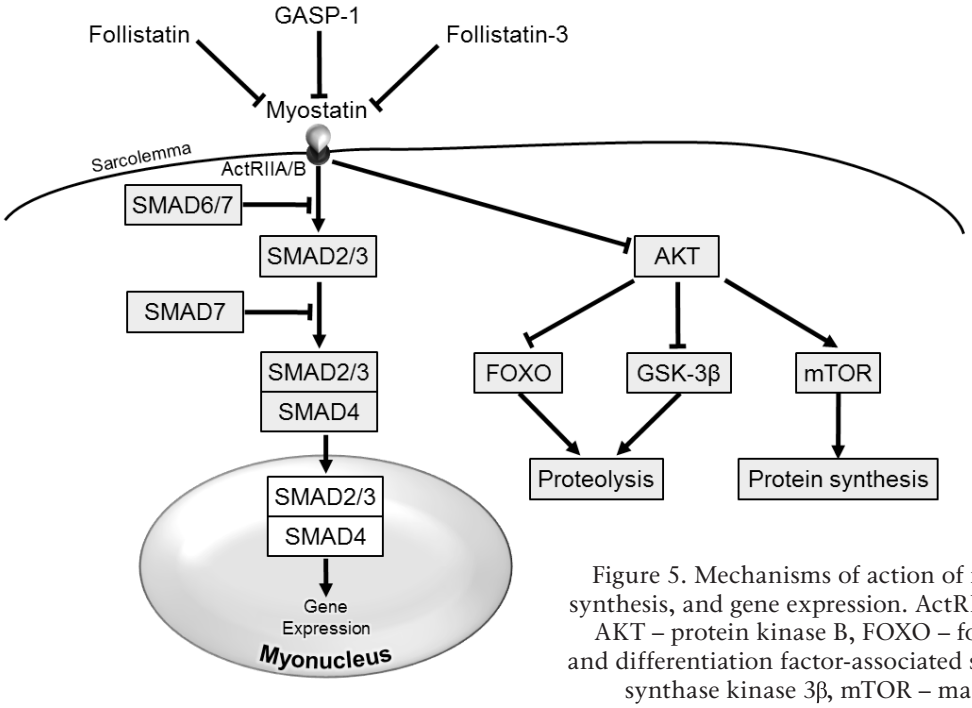

Figure 5. Mechanisms of action of myostatin on proteolysis, protein synthesis, and gene expression. ActRIIA/B – type IIA/B activin receptor, AKT – protein kinase B, FOXO – forkhead box O, GASP-1 – growth and differentiation factor-associated serum protein 1, GSK-3β – glycogen synthase kinase 3β, mTOR – mammalian target of rapamycin, SMAD – MAD-related protein

(NF-κB) activity is related to an increase in the expression of MuRF-1 but not of MAFbx [67] and this pathway could present increased activity during exercise owing to ROS production [68], which could explain the increase in mRNA expression of MuRF-1.

In summary, TVO can affect myostatin pathway activity through decreasing the expression of its mRNA and/or the protein itself; increasing the expression of mRNA and/or inhibitory factors of this pathway, such as follistatin, SMAD-7, and GASP-1; and promoting increased activity of factors related to the development of muscle tissue, such as MyoD, by decreasing the activity of this pathway (Figure 5).

Heat shock proteins

Heat shock proteins (Hsp) are a highly conserved group of polypeptides that are rapidly synthesized by cells of all organisms, from bacteria to humans, in response to a variety of stressors [69]. Several proteins in this set have been identified and are grouped into families depending on their molecular mass in kilodaltons (kDa) [9, 69, 70]. It is generally accepted that 'Hsp' refers to a specific protein and 'HSP' corresponds to a family of Hsps [70].

According to Liu et al. [70], among Hsps, the most prominent in skeletal muscles are: 1) small Hsps (Hsps with the molecular mass of 8–27 kDa), which play an important role in facilitating the targeting and removal of denatured proteins, muscle contractions (especially slow twitch muscle fibres), microfilament stabilization, transduction of cytokine signals, and the process of protein synthesis and muscle development; 2) HSP90, a family comprising 3 proteins including two cytoplasmic isoforms, Hsp90α and Hsp90β, and the glucose-regulated protein (GRP94), in which Hsp90 plays a role in the regulation of steroid hormone receptor activity; and 3) HSP70, which is a family of heat shock proteins composed of 4 principal forms, 72 kDa, 73 kDa, 75 kDa and 78 kDa, being that the proteins of 75 kDa (GRP75) and 78 kDa (GRP78) are not specifically induced by heat shock, but by deprivation of glucose, calcium influx, or glycolysis disturbing agents. The proteins with 72 kDa or inducible Hsp70 can be the most abundant Hsps induced in cells in response to stress and therefore the most commonly studied form of Hsp among the heat shock proteins [70]. Because of its essential and universal role in maintaining cellular homeostasis, Hsp72 serves as a chaperone involved in many cellular processes, so that it can have a profound impact on protein *turnover*, energy metabolism, muscle function, muscle regeneration, hypertrophy, and adaptation [70].

The Hsps have been demonstrated to have their transcription induced by hyperthermia, ischemia-reperfusion injury, hypoxia, energy depletion, acidosis, and ROS formation, being that these stimuli are similar to the integrated metabolic changes associated with exercising [69]. In fact, strength exercises have been shown to exert a strong impact on heat shock proteins [69–75]. Paulsen et al. [75] demonstrated that a session of maximum eccentric exercise in untrained individuals promoted a rapid and transient binding and accumulation of Hsp27 and αB-crystallin (a small Hsp) in myofibrils during exercise, while the synthesis of Hsp72 increased in the hours and

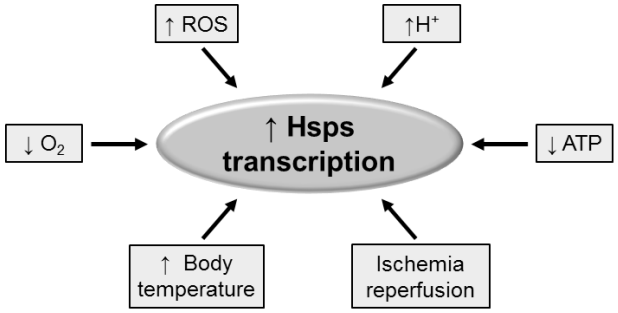

Figure 6. Factors that increase the transcription of Hsps. ATP – adenosine triphosphate, Hsps – heat shock proteins, ROS – reactive oxygen species

days after stimulation. However, intense strength training in the long term, both concentric and eccentric, in trained individuals can attenuate the response of Hsps (Hsp72, GRP75, and ubiquitin [small Hsp]) [71]. However, the study of Liu et al. [74] demonstrated that high intensity resistance training induced an increase in Hsp72, with a discrepancy between protein expression and the increase in mRNA expression in well-trained rowers.

The levels of Hsp72, Hsp27, and GRP75 increase in long term exercise in untrained individuals in both high and low intensity and high and low volume activities [72]. Moreover, Gjøvaag and Dahl [72] observed that volunteers who performed more repetitions presented lower post-training values than individuals who performed fewer repetitions, and subjects with lower pre-training values responded with greater changes in post-training levels of Hsps, while high pre-training levels demonstrated a negative development in the post-training values. Long term training composed of different forms, durations, and intensities has also been shown to induce increased levels of Hsp72 in trained rowers [73]. Thus, many variables can affect the responses of Hsp to training, such as intensity, volume, degree of training, and there is a very complex interaction between these variables, as part of adaptive mechanisms to exercise [70].

The surgical restriction of venous blood flow in rats has been shown to increase Hsp72 content in the plantar muscle 14 days after the surgery [15]. As Hsp72 relates to adaptations to exercise [69, 70], Kawada and Ishii [15] proposed that the increased production of Hsp72 may play a role in muscle hypertrophy observed through its hypertrophic or anti-apoptotic effect [70]. However, Fry et al. [1] found no change in the total content of HSP70 after a TVO session in older men. Perhaps the lack of effect of TVO on the expression of total HSP70 could be due to the fact that aging negatively influences the expression of heat shock proteins [69, 70]. Thus, TVO could have an impact on Hsps, which, owing to their actions in adaptations to exercise, may be an important point in the responses to this methodology. Figure 6 summarizes the factors that affect Hsps transcription.

Nitric oxide synthase

Nitric oxide synthase (NOS) is an enzyme that has three isoforms (neuronal NOS [nNOS or NOS I], induced NOS [iNOS or NOS II], endothelial NOS [eNOS or NOS III]) responsible for the production of nitric oxide (NO) [76]. In mammals, the greatest source of nNOS in terms of tissue is skeletal muscle mass [76]. In fact, the skeletal muscle of all mammals can express any of the 3 NOS isoforms, depending on factors such as age, stage of development, innervation and activity, cytokine exposure history and growth factors, muscle fibre type and species [77]. The enzymatic activity of nNOS and eNOS is regulated by Ca^{2+} and calmodulin [76, 77]. iNOS remains bound to calmodulin even at basal calcium levels [76, 77]. In muscle, nNOS is expressed by muscle activity, the aging process, and 'crushing' injuries; iNOS is expressed primarily by cytokines; and eNOS is expressed by chronic stimulation [77]. Thus, exercise, both acute and chronic, is a triggering factor of the enzymatic activity of NOS, also affecting the expression of its isoforms.

NO, among many other factors, has been shown to mediate the activity of satellite cells [78] and blood flow [77]. Activated satellite cells proliferate and differentiate, and may take two different destinations: fusing to damaged muscle fibres to aid in recovery (hypertrophy) or merging into each other, forming a new myofibre (hyperplasia) [78]. One of the few studies that examined the expression of some isoforms of NOS and NO production during blood flow restriction is that of Kawada and Ishii [15]. It demonstrated that rats with surgically obstructed venous circulation of the right hindlimb presented increased expression of NOS I in the plantar muscle but without an increase in NO concentration 14 days after the operation. The authors proposed that because the concentration of NO was indirectly measured by its oxidation products, the values obtained could have resulted from the production and breakdown of NO, both of which can be influenced by the occlusion of blood flow.

Accordingly, TVO could increase the expression of NOS and, consequently, the production of NO, which has some influence on the process of protein synthesis by affecting the activity of satellite cells and blood flow (Figure 7). However, there are few studies to date that have analysed these factors and their role in hypertrophy derived from TVO.

Conclusions

On the basis of the above considerations, among the principle adaptive mechanisms that have been raised to explain the effects of TVO, the following are proposed: 1) preferred or additional recruitment of fast-twitch muscle fibres; 2) longer duration of metabolic acidosis derived from the holding back and accumulation

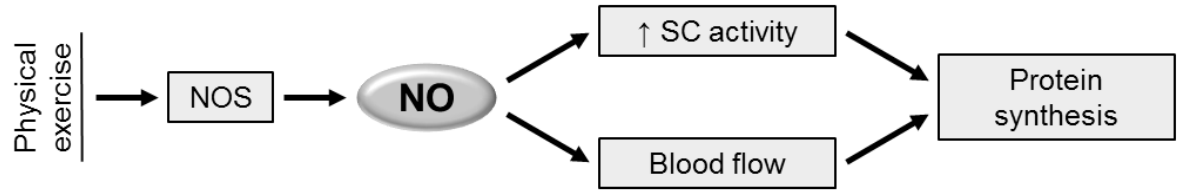

Figure 7. Illustration demonstrating the influence of exercise on NOS and NO and the physiological effects. NOS – nitric oxide synthase, SC – satellite cells

of protons H^+; 3) alterations in contractile and mechanical deformations in the sarcolemma arising from the *cuffs*; 4) metabolic adaptations of the glycolytic system; 5) production of ROS; 6) reactive hyperaemia after removing the external pressure; and 7) myogenic satellite cell activation [9]. It is evident that there is no consensus about which of these mechanisms is responsible for the main adaptations from TVO, although most probably all the above mechanisms contribute, each in its own way, to the effects of this training strategy.

It is worth mentioning that there has been little investigation into some of the effects of this training, for example on the circulatory system. Within the framework of sports training, we discuss the likely benefits that this training methodology could provide to athletes; however, sport-specific questions require further investigation before incorporating *KAATSU training* in sports preparation.

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