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REVIEW ARTICLE

A Review on the Mechanisms of Blood-Flow Restriction Resistance Training-Induced Muscle Hypertrophy

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Abstract It has traditionally been believed that resistance training can only induce muscle growth when the exercise intensity is greater than 65 % of the 1-repetition maximum (RM). However, more recently, the use of low-intensity resistance exercise with blood-flow restriction (BFR) has challenged this theory and consistently shown that hypertrophic adaptations can be induced with much lower exercise intensities (<50 % 1-RM). Despite the potent hypertrophic effects of BFR resistance training being demonstrated by numerous studies, the underlying mechanisms responsible for such effects are not well defined. Metabolic stress has been suggested to be a primary factor responsible, and this is theorised to activate numerous other mechanisms, all of which are thought to induce muscle growth via autocrine and/or paracrine actions. However, it is noteworthy that some of these mechanisms do not appear to be mediated to any great extent by metabolic stress but rather by mechanical tension (another primary factor of muscle hypertrophy). Given that the level of mechanical tension is typically low with BFR resistance exercise (<50 % 1-RM), one may question the magnitude of involvement of these mechanisms aligned to the adaptations reported with BFR resistance training. However, despite the low level of mechanical tension, it is plausible that the effects induced by the primary factors (mechanical tension and metabolic stress) are, in fact, additive, which ultimately contributes to the adaptations seen with BFR resistance training. Exercise-induced mechanical tension and metabolic stress are theorised to signal a number of mechanisms for the induction of muscle growth, including increased fast-twitch fibre recruitment, mechanotransduction, muscle damage, systemic and localised hormone production, cell swelling, and the production of reactive oxygen species and its variants, including nitric oxide and heat shock proteins. However, the relative extent to which these specific mechanisms are induced by the primary factors with BFR resistance exercise, as well as their magnitude of involvement in BFR resistance training-induced muscle hypertrophy, requires further exploration.

Key Points

Mechanical tension and metabolic stress are both primary mechanisms of resistance training-induced muscle hypertrophy.

Metabolic stress may play the dominant role in mediating the potent hypertrophic effects seen with blood-flow restriction (BFR) resistance training, but mechanical tension also plays a part.

Mechanical tension and metabolic stress act synergistically to mediate numerous secondary associated mechanisms, all of which stimulate autocrine and/or paracrine actions for the induction of muscle hypertrophy with BFR resistance training.

1 Background

During resistance exercise, motor units, and hence muscle fibres, are recruited according to the 'size principle' [1], in which the smaller motor units associated with type I

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muscle fibres are activated initially at low intensities, and the larger motor units associated with type II muscle fibres are recruited at higher exercise intensities with increasing level of contractile force. In order to increase muscle mass and strength, it is important to activate type II muscle fibres during training, since these fibres have been shown to be more responsive to hypertrophy than type I fibres [2, 3] and are generally larger. Therefore, previously it has been suggested that only moderate–high-intensity resistance exercise with intensities >65 % of the 1-repetition maximum (RM) can induce significant gains in muscle mass and strength [4, 5].

However, more recent research has demonstrated the effectiveness of exercise training with blood-flow restriction (BFR), which can produce hypertrophic adaptations with much lower exercise intensities than previously believed [6–17]. In particular, most studies appear to have utilised a low-intensity (<50 % 1RM) resistance exercise protocol with BFR [6–11] for the induction of muscle hypertrophy, although some have also shown the utility of a low-intensity walking intervention (2-min bouts at 50 m/min) [14]. The BFR in such exercise protocols is typically achieved by restricting blood flow to the muscle with the application of external pressure via a tourniquet [18], pressurised cuff [19], or elastic banding [20] that is applied over the proximal portion of the upper or lower extremities. It has been suggested that the external pressure applied is sufficient to maintain arterial inflow whilst occluding venous outflow of blood distal to the occlusion site [16], although here, it is difficult to envisage sufficient arterial inflow, since such restricted venous return is likely to reduce inflow of blood to the muscle. This reduced blood flow is thought to induce an ischemic/hypoxic environment that enhances the training effect in exercising muscle, leading to increased muscle mass and strength [6–10, 14].

Despite the fact that the robust effects of BFR resistance training in producing muscle hypertrophy have previously been documented by numerous studies [6–17], the underlying mechanisms responsible for such effects remain poorly understood. The resultant hypertrophic effects of resistance training with BFR have been primarily attributed to increased levels of metabolic stress (i.e., build-up of metabolites as a result of the ischemic/hypoxic environment) [21], which is theorised to induce muscle growth by acting on other factors, including the increased recruitment of fast-twitch muscle fibres [22, 23], elevations of systemic hormones [24, 25], cell swelling [26], and increased production of reactive oxygen species (ROS) [16, 27]. However, it must be noted that some of these mechanisms (i.e. increased recruitment of fast-twitch muscle fibres and ROS production) are not activated to the greatest extent by metabolic stress, and are more associated with high levels of mechanical tension (another primary factor of muscle growth) as that seen with high-intensity resistance training [19, 28–30], which perhaps questions their level of contribution in BFR resistance training-induced hypertrophy, given its low-intensity nature.

Despite the low level of mechanical tension, it is possible that the effects induced by the primary factors (mechanical tension and metabolic stress) are in fact additive, which ultimately contributes to the adaptations seen with BFR resistance training. However, the specific extent to which these primary factors activate the particular mechanisms for the induction of muscle growth with BFR resistance exercise, as well as their magnitude of involvement to BFR resistance training-induced muscle hypertrophy, is largely unknown. This topic is obviously very complex, and further exploration is required to provide a better understanding of the potential relative contributions of the mechanisms involved.

Thus, it is the purpose of this article to review the existing literature and explore in detail how muscle growth is induced with BFR resistance training, with particular regard to the relative contribution of mechanical tension and metabolic stress, as well as their associated mechanisms.

2 Literature Search Methodology

The National Library of Medicine (PubMed) database was used to search for relevant articles between January 2000 and June 2014. The specific search terms used in isolation and/or combination were ‘occlusion training’, ‘blood flow restriction’, ‘muscle hypertrophy’, ‘human’, ‘skeletal muscle’, ‘molecular signalling’, ‘kaatsu training’, ‘resistance training’, ‘adaptation’, ‘mechanical loading’, ‘metabolic stress’, ‘hormones’, ‘cellular’, and ‘anabolic hormones’. Reference lists of articles obtained from this search were also examined for additional relevant articles. The inclusion/exclusion criteria for studies were based on their potential relevance to the acute and/or chronic responses of resistance exercise with BFR. In addition, studies utilising other modalities of exercise with BFR were also considered if relevant information with regards to the mechanisms of hypertrophy with BFR resistance exercise was detailed.

3 Modes of Action

The mechanisms suggested to stimulate muscle growth from exercise-induced metabolic stress and/or mechanical tension include increased fast-twitch fibre recruitment, mechanotransduction, muscle damage, systemic and localised hormone production, cell swelling, and the production of ROS and its variants, including nitric oxide

(NO) and heat shock proteins [11–13, 20–29, 31, 32]. It is plausible that mechanical tension and metabolic stress activate similar mechanisms to promote hypertrophy and thus the effects may be additive and synergistic; however, it seems reasonable that some of these mechanisms would be more mediated (activated to a greater degree) by mechanical tension (i.e. fast-twitch fibre recruitment) and others by metabolic stress (i.e. systemic hormone production). To speculate, it is possible that the magnitude of contribution of the primary factors and their associated mechanisms in producing muscle hypertrophy actually depends on the training intensity/modality employed. For instance, high-intensity resistance exercise may induce a higher level of mechanical tension and a lower level of metabolic stress than moderate-intensity resistance exercise and low-intensity resistance exercise with BFR [4, 33, 34], whereas low-intensity BFR resistance exercise may induce a lower degree of mechanical tension but a higher level of metabolic stress than moderate- and high-intensity resistance exercise [14, 24, 35]. Thus, moderate-intensity resistance exercise may induce an optimal combination of both mechanical tension and metabolic stress, perhaps lending itself to the greatest hypertrophic potential (see Fig. 1).

Based on the above intensity/modality-specific mechanisms theory, metabolic stress appears to play the dominant role in mediating muscle hypertrophy with BFR resistance training. However, it would still seem of significant importance to not categorically exclude the potential role of mechanical tension, since it would most likely be this combination of complex cascades that ultimately contributes to muscle growth.

4 Primary Mechanisms

The following sub-sections discuss the primary factors in greater detail with respect to their relative contribution to BFR resistance training-induced hypertrophy.

4.1 Mechanical Tension

A large body of research indicates that mechanical tension acts as a primary mechanism of muscle growth. This was first noted by Goldberg et al. [36] where induced mechanical "strain" on muscle was found to attenuate the atrophy caused by unloading, suggesting that mechanical tension is a critical factor initiating compensatory muscle growth. Subsequently, Spangenburg et al. [37] reported an increased mechanical load to induce muscle hypertrophy in a rat model, and Vandenburgh and Kaufman [38] also reported mechanical stretch to be an important factor for hypertrophy using an in vitro model.

The mechanisms put forward by which mechanical tension induces muscle hypertrophy include mechanotransduction [31, 39, 40], increased localised hormone production [41], muscle damage [42], ROS production [42, 43] and increased fast-twitch fibre recruitment [17, 28, 30]. All of the above have been reported to increase protein synthesis through activation of signalling pathways [44, 45], and/or satellite cell activation and proliferation [41] for the induction of muscle growth. Although it can be argued that the low level of mechanical tension associated with BFR resistance exercise would not induce these mechanisms to any great extent, metabolic stress has also been shown to mediate similar mechanisms, and as such the effects may be additive.

4.2 Metabolic Stress

Metabolic stress (i.e. accumulation of metabolites during exercise) has been reported as being equally as important as mechanical tension, if not more, for the induction of muscle growth [9, 12, 28, 31, 46]. To illustrate, Goto et al. [47] compared the acute and chronic effects of two volume/intensity-matched resistance exercise protocols (3–5 sets of 10 reps at 75 % 1RM), with the only difference being that one protocol included a 30-s rest period in the midway point of each set to try and reduce the degree of metabolic build-up, whereas the other did not. Results showed blood lactate concentrations to be significantly higher following the without-rest protocol relative to the with-rest protocol. Additionally, following 12 weeks of training, the without-rest regimen was found to significantly increase muscle cross-sectional area (CSA), whereas no such differences were observed following the with-rest protocol, indicating a direct link between metabolic stress and muscle hypertrophy.

Indeed, such levels of metabolic stress are also magnified under ischemic/hypoxic conditions as that seen during BFR resistance exercise [24, 35]. Blood lactate concentrations have previously been shown to be significantly higher following low-intensity resistance exercise performed under ischemic conditions such as BFR [24] and hypoxia [35] compared with the same exercise protocol performed under normal conditions. The potential hypertrophic effects of the metabolic stress associated with BFR resistance exercise have also been demonstrated by numerous studies where a period of low-intensity resistance exercise ($\sim$30–50 % 1RM) with BFR ($\sim$110–200 mmHg) was found to result in a significantly greater increase in muscle CSA than the same training programmes performed without BFR [6–8]. In addition, direct relationships between other indices of metabolic stress (Pi and intramuscular pH) and muscle hypertrophy following a period of low-intensity (20 % 1RM) resistance

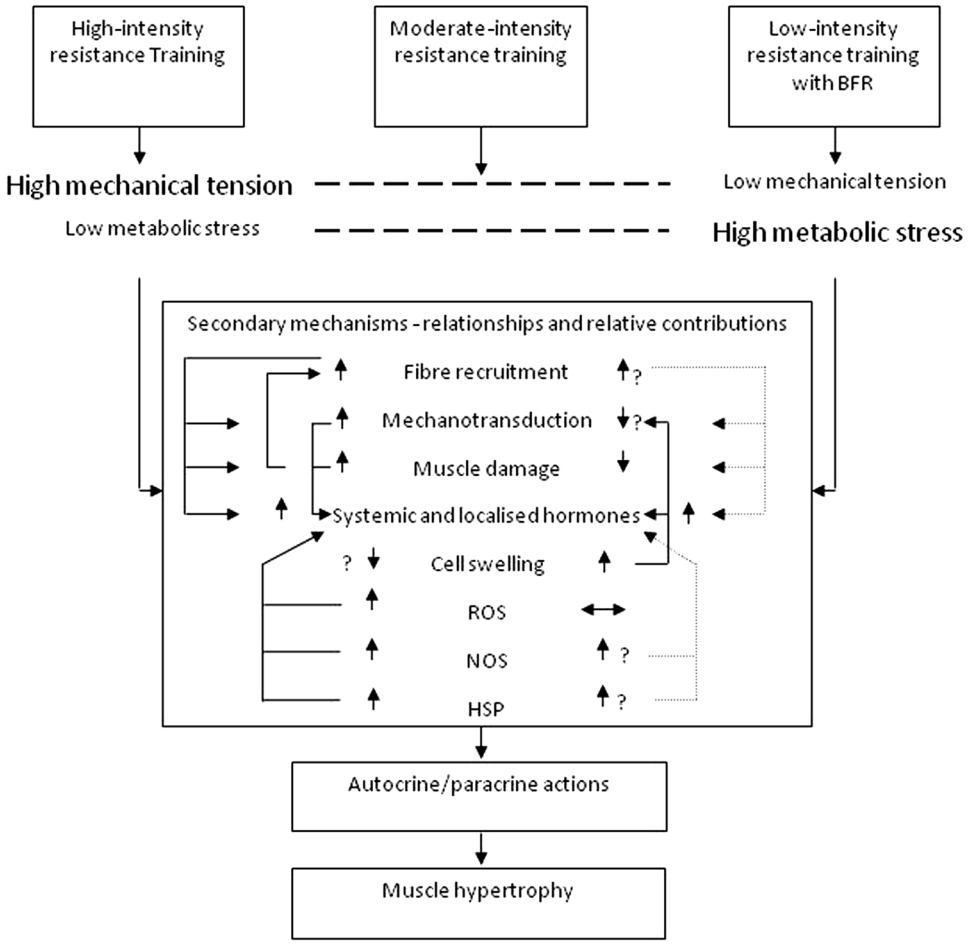

Fig. 1 The relative contributions of mechanical tension and metabolic stress in mediating muscle hypertrophy, dependant on the training intensity and/or modality. *Arrows* highlight potential degrees of activation of resultant intermediate secondary mechanisms and their possible relationships. *Vertical arrows* (↕) represent higher/lower degree of activation, *horizontal arrows* (↔) represent no effect, *interconnecting arrows* represent potential relationships between secondary mechanisms, *dotted interconnecting arrows* indicate equivocal relationships. *BFR* blood flow restriction, *HSP* heat shock proteins, *NOS* nitric oxide synthase, *ROS* reactive oxygen species

exercise with BFR have also been reported elsewhere in the literature [9]. This perhaps highlights the prominent role of metabolic stress in mediating hypertrophic adaptations following resistance training with BFR. It has been theorised that exercise-induced metabolic stress mediates muscle hypertrophy via a number of mechanisms, including elevated systemic hormone production [25], increased fast-twitch fibre recruitment [6, 7], cell swelling [26], muscle damage [31, 48] and increased production of ROS [13, 27, 31, 49], all of which are thought to mediate muscle protein signalling and/or satellite cell proliferation for the induction of muscle growth.

5 Secondary Mechanisms

As outlined earlier, the primary factors are expected to act on a number of associated secondary mechanisms for the induction of muscle growth. The following sub-sections discuss these secondary factors in greater detail in terms of their extent of activation by mechanical tension/metabolic stress and their magnitude of involvement in BFR resistance training-induced hypertrophy.

5.1 Mechanotransduction

Mechanical tension leads to morphological adaptations through the process of mechanotransduction, whereby sarcolemmal-bound mechanosensors, such as integrins and focal adhesions, convert mechanical energy into chemical signals that mediate intracellular anabolic and catabolic pathways, ultimately leading to a shift in muscle protein balance that favours synthesis over degradation [40]. Baar and Esser [45] reported increased phosphorylation of p70S6 kinase (p70S6k) following high-resistance lengthening contractions, which also correlated to percent increases in muscle mass ($r = 0.998$). This process of mechanotransduction could in theory occur at the level of

the lipid bilayer and/or at the matrix of the integrin cytoskeleton [50]. It has been proposed that, during damage or repair to the lipid bilayer, vesicle plugs can form whereby intracellular components can fuse with the bilayer and release insulin-like growth factor (IGF)-1, which ultimately up-regulates protein synthesis via activation of phosphoinositide 3-kinase (PI3K)/Akt [51]. Other associated mechanisms here include changes in the permeability of the bilayer by stretch, leading to activation of G proteins and subsequent muscle hypertrophy [38]. In addition, the mechanical stretch can also increase production of neuronal NO in muscle fibres, causing release of intracellular calcium, which has also been shown to activate the mammalian target of rapamycin (mTOR) signalling pathway [49], promoting muscle anabolism.

Collectively, there is a large body of research emphasising mechanotransduction as an important mechanism of muscle hypertrophy. However, no evidence yet exists with regards to its potential contribution to the training-induced effects of resistance exercise with BFR; although it is questionable whether such mechanotransduction processes would contribute to BFR resistance training-induced hypertrophy given its low mechanical stress nature.

5.2 Muscle Damage

Exercise-induced muscle damage (EIMD) is purported to be an essential regulator of satellite cell-mediated compensatory muscle growth (see later section) [52–54]. The greatest damage to muscle tissue is seen with eccentric exercise, where muscles are forcibly lengthened [55]. Thus, support for the potential anabolic role of EIMD perhaps stems from studies that have reported the hypertrophic response [56] to be blunted when the eccentric phase is omitted from training. In addition, a meta-analysis by Roig et al. [57] suggests that eccentric training is superior to concentric training in mediating muscle hypertrophy. Taken together, these studies support the notion that EIMD (eccentric exercise) is a potent stimulus for muscle growth. However, the 'repeated bout effect' phenomenon suggests that, although one bout of eccentric exercise may induce muscle damage, repeated bouts of the same exercise are not associated with such effects [58], which perhaps contradicts any association of EIMD to hypertrophy, as multiple exercise sets and chronic training are, in this sense, likely to lessen the muscle damage response.

It is currently unclear whether EIMD plays a role in the hypertrophic adaptations of BFR resistance exercise, as previous research is somewhat diverse. Through the use of indirect markers (i.e. maximal voluntary contraction [MVC] torque, muscle soreness), Thiebaud and colleagues [59, 60] showed BFR resistance exercise to induce only minimal levels of muscle damage (lasting less than 1 day), whereas Umbel et al. [61] have reported a sufficient degree of EIMD by BFR resistance exercise (lasting 48 h post-exercise). These discrepancies could perhaps be accounted for by differences in methodologies between the studies, including the prescribed exercise intensity, volume, and time under BFR [59–61].

Additionally, direct markers such as interleukin (IL)-6, which may provide a more accurate reflection of EIMD, have also been examined in response to BFR resistance exercise, with studies showing no increases [11, 62]. Although some studies [24, 63] have reported a gradual increase in IL-6 following low-intensity resistance exercise with BFR (110 mmHg; 214 mmHg), the overall effect sizes were very small, with levels reaching only one-quarter of those reported in response to high-intensity eccentric exercise [64]. These findings perhaps suggest that only high mechanical tension-associated exercise can induce the sufficient amount of muscle damage required for the production of IL-6 and subsequent compensatory muscle growth. Thus, EIMD may not contribute to BFR resistance training-induced hypertrophy, due to its low-intensity nature.

5.3 Systemic and Localised Hormones

Another popular theory proposed by several researchers to explain the hypertrophic effects of BFR resistance training is that the increased metabolic stress triggers a strong anabolic hormonal response post-exercise [24]. Numerous studies have reported low-intensity resistance exercise with BFR to facilitate the expression of many systemic hormones, including growth hormone (GH) [24, 25, 65] and IGF-1 [19], although the latter is not consistent in all trials [65]. However, it must be noted that such increases in systemic hormones do not appear to be associated with increased muscle protein synthesis [66–69] or long-term hypertrophic adaptations [70]. West and Phillips [69] reported an increase in myofibrillar protein synthesis ($\sim$78 %) in response to a resistance exercise protocol (unilateral elbow flexion), independent of any changes in the systemic levels of GH, IGF-1 and testosterone, respectively. Also, Mitchell et al. [70] found 16 weeks (four sessions per week) of resistance training to significantly increase muscle fibre CSA of the vastus lateralis ($\sim$20 %) as well as leg press strength ($\sim$61 %), without any associated increases in GH, free testosterone and IGF-1.

Conversely, mechanical tension-induced localised hormones may in fact contribute to such hypertrophic adaptations. To illustrate, an animal model in which the IGF-1Ea (systemic form) receptor was knocked out demonstrated that animals were still able to undergo muscle hypertrophy [37]. This could be attributable to the production of the localised IGF-1 isoform, IGF-1Ec, better known as mechano-growth factor (MGF), which is

believed to be principally responsible for the hypertrophic effects with resistance training, as opposed to the systemic forms of IGF-1 (IGF-1Ea and IGF-1Eb) [21, 71]. Although each of these isoforms is expressed in muscle tissue [72], only IGF-1Ec appears to be locally activated by mechanical stimuli and cellular damage [73, 74]. Because of its rapid expression following mechanical loading, MGF is thought to help 'kick start' the post-exercise hypertrophic response and facilitate local repair of damaged tissue [75]. MGF gene expression is thought to carry out signalling through multiple anabolic cascades including mTOR [76], mitogen-activated protein kinase (MAPK) [77], and various calcium-dependent pathways [78], thereby directly mediating muscle protein synthesis. In addition, MGF could also induce muscle growth through satellite cell activation, proliferation, and differentiation [79, 80], highlighting its utility in autocrine and paracrine actions.

However, the extent to which these mechanical tension-induced localised factors exist following resistance exercise with BFR and contribute to the hypertrophic adaptations seen with BFR resistance training is yet to be elucidated.

5.4 Cell Swelling

One of the more novel mechanisms involved in the hypertrophic adaptations of BFR resistance exercise has been reported to be the increase in intracellular hydration, a phenomenon known as 'cell swelling'. Previously, it has been reported that hydration-mediated cell swelling results in an increase in protein synthesis and a decrease in proteolysis in a variety of different cell types, including hepatocytes, osteocytes, breast cells and muscle fibres [81].

Increased accumulation of metabolites via BFR creates a pressure gradient favouring the flow of blood into the muscle fibres (intracellular space). The resulting enhanced reperfusion and subsequent intracellular swelling is believed to threaten the structural integrity of the cell membrane [26], which causes the cell to initiate a signalling response that chronically leads to a reinforcement of its ultrastructure [32, 82]. There is evidence that signalling is carried out via integrin-associated volume osmosensors within cells [83]. The sensors, in turn, activate anabolic protein kinase transduction pathways, possibly mediated by autocrine effects of growth factors [84, 85]. Research indicates that anabolic functions are carried out in an mTOR-independent fashion [86], with MAPK modules being the primary mediator of swelling-induced anabolism [87, 88], although it has been reported that cell swelling could also induce muscle growth through the proliferation and fusion of satellite cells [89]. However, Gundermann et al. [90] recently reported no significant increases in muscle protein synthesis following a simulation of the reactive hyperaemia response via a pharmacological vasodilator, suggesting that reperfusion may not be responsible for the hypertrophic adaptations of BFR resistance exercise.

Research is currently very scarce concerning the potential contribution of cell swelling to hypertrophic adaptations and so any definitive statements at this time would be premature. Also in question is whether cell swelling is solely mediated by metabolic stress or whether mechanical tension also plays a part.

5.5 Reactive Oxygen Species

The acute production of ROS by muscles during exercise [91] is believed to be an important mechanism mediating post-workout anabolic adaptations [92–95]. ROS production has been shown to promote growth in both smooth and cardiac muscle [96], and previous work also suggests that it may play a role in the hypertrophic effects of BFR resistance training [6, 43, 97], since hypoxia and subsequent reperfusion is thought to further heighten the production of ROS [98, 99]. However, evidence supporting its potential contribution to BFR resistance training-induced hypertrophy is conflicting. Although hypoxia and subsequent reperfusion associated with arterial occlusion has been shown to increase ROS production [98, 99], Takarada et al. [24] and Goldfarb et al. [29] both reported no significant increases in markers of ROS (lipid peroxide and protein carbonyl) following a low-intensity resistance exercise protocol with BFR. These disparate findings could perhaps be explained by differences in the applied length of the BFR stimulus between the studies. Most BFR resistance exercise protocols last 5–10 min [24, 29], and as such may not elevate ROS levels to the same extent as longer occlusive stimuli (4 h) [98].

Interestingly, previous work appears to support the notion that mechanical load is the dominant factor responsible for the production of ROS, as opposed to metabolic stress. Goldfarb et al. [29] compared the ROS responses between volume-matched moderate-intensity resistance exercise and low-intensity resistance exercise with BFR and found plasma protein carbonyl levels and blood glutathione ratio (markers of ROS) to be significantly greater following the moderate-intensity resistance exercise protocol, suggesting that mechanical tension plays the dominant role in generating ROS. Hence, it is perhaps not surprising that previous studies utilising BFR resistance exercise protocols have reported no significant increases in ROS [24, 29], as these typically involve low levels of mechanical tension ($\sim$20 % 1RM) and thus work.

5.6 Nitric Oxide

A particular variant of ROS that has been linked to compensatory muscle hypertrophy is NO, an important cellular

signalling molecule produced constitutively at high levels in muscle by neuronal nitric oxide synthase (NOS)-1 [100–102]. Previous research indicates that NO can stimulate satellite cell activation and proliferation [103], possibly via synthesis of hepatocyte growth factor [42]. Interestingly, NO production has also been shown to directly mediate protein synthesis through the activation of the transient receptor potential cation channel, subfamily V, member 1 (TRPV1) within the sarcoplasmic reticulum via peroxynitrite-dependent mechanisms, resulting in mTOR activation and subsequent protein synthesis [104].

NO production appears to be primarily increased in response to high mechanical tension [105], and so, it seems unlikely that NO would play a part in BFR resistance training-induced hypertrophy given its low-intensity nature. However, there is evidence suggesting a potential increase in NO production with a BFR resistance exercise protocol. A number of studies have reported low-intensity BFR resistance exercise to increase conduit-artery maximal dilation [106, 107], which itself is dependent on NO production [108]. In fact, the contribution of NO to conduit-artery vasodilation is enhanced under ischemic/hypoxic conditions compared with normoxic conditions [109, 110], augmenting the up-regulation of endothelial NOS (eNOS). This, combined with the protective effects of ischemic preconditioning [111, 112] has been shown to contribute to an increase in NO bioavailability [113]. In addition, Kawada and Ishii [27] have also reported an increased expression of NOS-1 following 2 weeks of chronic occlusion in an animal model. Thus, NO production may in fact be evident with BFR resistance exercise, which could potentially contribute to hypertrophic effects via autocrine [104] and/or paracrine [105] actions.

5.7 Heat Shock Proteins

ROS may also indirectly influence anabolism by mediating transcription of highly conserved stress proteins called heat shock proteins (HSPs). Under normal physiological conditions, HSPs act as chaperones, aiding in the assembly and translocation of proteins [114], but when the body is subjected to stress, they are thought to play a role in modulating the effects of the stress to maintain cellular homeostasis (i.e. limiting oxidative damage caused by ROS production) [115]. It should be noted that, in addition to ROS-mediated transcription, HSPs are also induced by heat, hypoxia, acidosis, and ischemia–reperfusion [116], which may suggest that metabolic stress can also regulate HSP activity, similarly to mechanical tension-induced ROS.

Kawada and Ishii [27] first reported that HSP72 was significantly elevated in the plantaris muscle of rats following 2 weeks of exercise with BFR. These findings were associated with a significant increase in muscle hypertrophy, suggesting that HSPs may contribute to the hypertrophic adaptations of BFR resistance exercise. However, Fry et al. [62] reported no significant increases in HSP70 content following a low-intensity (20 % 1RM) resistance exercise protocol with BFR (200 mmHg). These conflicting data could perhaps be accounted for by the different HSPs examined. It is possible that only certain HSPs (HSP72) are increased with BFR exercise, whereas others are not (HSP70), and, as such, only specific HSPs may play a role in hypertrophy. Further research is clearly required on varying HSPs to identify the specific HSP isoforms that may have an important post-exercise anabolic role.

5.8 Fibre Recruitment

The increased recruitment of type II muscle fibres with BFR resistance exercise has been proposed to be a critical factor responsible for the potent hypertrophic effects [21, 117]. According to the size principle for neuromotor control [1], fast-twitch muscle fibres are only recruited at higher exercise intensities. However, BFR resistance training research has demonstrated that recruitment of fast-twitch muscle fibres is possible even at very low intensities, likely due to the inadequate oxygen supply for slow-twitch fibres and high metabolite accumulation [22, 117, 118]. Both reduced oxygen and metabolite accumulation can increase fibre recruitment, mechanistically speaking, through the stimulation of group III and IV afferents, which may cause inhibition of the alpha motorneuron, resulting in an increased fibre recruitment to maintain muscular force and protect against conduction failure [21, 119]. This is also supported by many reports in the literature showing higher motor unit recruitment/firing frequency and activation of fast-twitch muscle fibres via electromyography (EMG) during low-intensity BFR resistance exercise, relative to the same exercise protocol without BFR [6, 7, 21–23]. Indeed, such increased electrical activity could stimulate muscle protein synthesis via the transcriptional ca^{2+}/calmodulin—phosphatase calcineurin and/or the ca^{2+}/calmodulin-dependant kinase pathways [120].

However, increased recruitment of fast-twitch muscle fibres may not always be observed with BFR resistance exercise, since Wernbom et al. [97] and Kacin and Strazar [121] both reported similar levels of quadriceps EMG activity during low-intensity knee extension exercise with and without BFR. It is also important to note that BFR resistance exercise does not necessarily recruit as many fast-twitch muscle fibres as high-intensity resistance exercise [17, 28, 30], which may suggest that mechanical tension plays a greater role than metabolic stress in mediating fast-twitch fibre recruitment. However, taken together, it would seem that increased fast-twitch fibre recruitment is

responsible for at least some of the hypertrophic adaptations seen with BFR resistance training.

6 Autocrine/Paracrine Actions

Muscle growth is ultimately brought about by autocrine (i.e. stimulation of protein synthesis through an increase in anabolic and/or decrease in catabolic signalling pathways) and/or paracrine (i.e. increased satellite cell activation, proliferation, and fusion) actions. The two primary mechanisms are thought to act on their associated secondary mechanisms that subsequently stimulate protein synthesis (autocrine) and/or satellite cell activity (paracrine), for the induction of muscle hypertrophy. The following sections discuss the potential autocrine and paracrine mechanisms involved in BFR resistance training-induced hypertrophy.

6.1 Autocrine: Protein Synthesis

6.1.1 IGF-1/PI3K/Akt/mTOR Signalling Pathway

The IGF-1/PI3K/Akt signalling pathway plays a key role in the regulation of muscle mass [44, 122, 123], and promotes muscle hypertrophy by stimulating overall protein synthesis and suppressing proteolysis. In skeletal muscle, activation of Akt by IGF-1 stimulates protein translation through the induction of mTOR, which is involved in the regulation of messenger RNA (mRNA) translation initiation and has been reported to play a significant role in exercise-induced muscle protein synthesis and training-induced hypertrophy [44, 45, 124, 125]. Low-intensity (20 % 1RM) resistance exercise with BFR (200 mmHg) has also been shown to stimulate the mTOR signalling pathway via its associated downstream effectors (ribosomal S6 kinase 1 [S6K1] and ribosomal protein S6 [rpS6] phosphorylation) [11, 62], highlighting its potential contribution to the potent effects of BFR resistance training. In addition, the enhanced mTOR signalling to S6K1 also inhibits the activity of eukaryotic translation elongation factor 2 (eEF2) kinase, which significantly reduces eEF2 phosphorylation [11] and thus promotes translation initiation and elongation [126].

6.1.2 Myostatin Smad2/3 Signalling Pathway

Myostatin is a member of the transforming growth factor (TGF)-β super-family that negatively regulates muscle growth [127–132] via the Smad2/3 phosphorylation-induced inhibition of myoblast and myotube differentiation [133–136].

Theoretically, any potential decrease in myostatin expression would indicate an increased signalling in favour of muscle hypertrophy. Previous research has also shown the expression of myostatin to be diminished in response to BFR resistance exercise, highlighting its potential contribution to training-induced effects [27, 137, 138]. Moreover, previous research has also demonstrated that decreased myostatin expression following 8 weeks of resistance exercise with BFR (20 % 1RM at 95 mmHg) is concomitant with increased muscle mass and strength (6.3 and 40 %) [138], thus emphasising the inhibitory role of myostatin in BFR resistance training-induced hypertrophy.

The specific mode of action by which myostatin is decreased with BFR resistance exercise may be attributable to the increased activation of mTOR, which has been shown to play an important role in regulating myostatin's inhibition of muscle growth [136]. However, increased mTOR activation may not be the only factor blunting myostatin-induced effects, as previous research has demonstrated that blocking mTOR activity does not fully prevent the increases in protein synthesis and hypertrophy phenotype associated with myostatin inhibition [139, 140]. Thus, other factors may also coexist with respect to inhibiting myostatin and promoting muscle growth. One such factor may be JunB transcription, which has also been associated with myostatin inhibition [141], but no research yet exists with respect to its potential activation with BFR resistance exercise.

6.1.3 FOXO Transcription Factors

One downstream target of the PI3K/Akt pathway is the Forkhead box O (FOXO) class of transcription factors, which interestingly enough has contrasting effects to the PI3K/Akt/mTOR pathway. Activation of the FOXO transcription factors has been shown to be associated with muscle wasting and the induction of muscle atrophy [142–144]. However, in growing muscles, FOXO transcription factors are maintained in an inactive state by phosphorylation via the PI3K/Akt signalling cascade [142]. In contrast, during atrophic conditions, the activity of the PI3K/Akt signalling pathway decreases, causing dephosphorylation of FOXO transcription factors and subsequent stimulation of muscle protein breakdown [145], via the ubiquitin–proteasome [142, 143] and autophagic/lysosomal pathways [146, 147] (see next sections). Based on these considerations, it appears that a suppression of FOXO transcription would in fact promote anabolism. The ability of the PI3K/Akt pathway to suppress the activation of FOXO transcription factors may therefore present another mechanism by which BFR resistance exercise induces hypertrophic adaptations. In other words, the Akt-induced activation of mTOR and its associated downstream targets may stimulate muscle protein synthesis, while the phosphorylation of FOXO transcription factors by Akt leave them inactive in the cytosol. Together, these changes may

lead to a positive muscle protein balance and ultimately muscle hypertrophy.

6.1.4 Ubiquitin–Proteasome Pathway

One mechanism by which FOXO transcription factors negatively regulate muscle growth is through activation of the ubiquitin–proteasome pathway [142, 143, 148, 149]. In particular, muscle RING finger-containing protein 1 (MuRF1) and muscle atrophy Fbox protein (MAFbx) are genes that encode for E3 ubiquitin ligases [150, 151]. Empirical evidence supporting their prominent catabolic roles is provided by Bodine et al. [150], who showed under atrophic conditions that mice null for either gene (MuRF1 or MAFbx) exhibit a resistance to muscle mass loss compared with wild-type controls, respectively.

MuRF1 induces muscle atrophy, at least in part, by directly ubiquitinating the thick filament of the sarcomere and causing the proteolysis of myosin proteins [152, 153], whereas MAFbx down-regulates protein synthesis via the ubiquitination of eIF3-f, a protein initiation factor [154]. Although no research currently exists with respect to the potential contribution of MuRF1 and MAFbx to BFR resistance training-induced hypertrophy, it is likely that any inhibition or decreased expression of these genes also plays some part in promoting hypertrophic adaptations. Interestingly, previous research has also shown that MuRF1 and MAFbx transcription can be at least partially inhibited by the activation of mTOR [155, 156], which has convincingly been shown to significantly increase in response to resistance exercise with BFR [11, 62]. It has been reported that mTOR activation blocks MuRF1 and MAFbx activity by inhibiting glucocorticoid activity [156], which is thought to synergise with FOXO transcription factors for the induction of these E3 ubiquitin ligases [157, 158]. Thus, it could perhaps be speculated that the increased activation of mTOR with BFR resistance exercise also inhibits MuRF1 and MAFbx activity to some degree, thereby promoting an increased signalling in favour of muscle hypertrophy.

6.1.5 Autophagic/Lysosomal Pathway

The other mechanism by which FOXO transcription factors induce muscle atrophy is via the autophagic/lysosomal system, which is independent of the ubiquitin–proteasome pathway [146, 147]. FOXO3 transcription, in particular, has been shown to stimulate autophagy in skeletal muscle for protein breakdown and atrophy [146] via a set of autophagy-related genes, including microtubule-associated protein 1 light chain 3 (LC3) and BCL2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) [146, 147]. FOXO3-induced LC3 up-regulation alone is not considered to be sufficient for triggering muscle autophagy [159, 160], but BNIP3 expression may, in fact, contribute to such muscle autophagic effects and act as a key mediator of FOXO3-induced atrophy [146, 161].

No information currently exists with regards to the potential role of the autophagic/lysosomal system in BFR resistance training-induced hypertrophy. However, it could perhaps be speculated once again that the potential activation of Akt/P13K in response to BFR resistance exercise would inhibit FOXO transcription to some extent [142], which in turn may blunt the BNIP3 response for muscle autophagy, ultimately increasing the potential for hypertrophy. This is indeed an attractive area for future research.

6.2 Paracrine: Satellite Cell Activity

Satellite cells are muscle-specific stem cells located under the basal lamina of muscle fibres and that are responsible for muscle regeneration [162]. They also contribute to the increase in the number of myonuclei during postnatal muscle growth [162] and compensatory muscle hypertrophy [163] by proliferating and fusing with the existing myofibres. Following EIMD, satellite cells undergo rapid proliferation, leading to subsequent muscle growth and remodelling. Multiple signals appear to trigger this activation, including the generation of sphingosine-1-phosphate in the inner side of the plasma membrane of the satellite cell, as well as NO production, which stimulates satellite cell activation, via increased activation of matrix metalloproteinases, ultimately leading to the release of hepatocyte growth factor from the extracellular matrix [42].

It seems unlikely that satellite cell mechanisms of muscle growth would be activated to a significant degree with BFR resistance exercise considering its low mechanical tension and minimal muscle damage-inducing nature [49]. However, interestingly, increases in satellite cell proliferation have been demonstrated in response to acute BFR resistance exercise in association with increased muscle protein synthesis [122] as well as chronic BFR resistance exercise concomitant with muscle hypertrophy [164], thus presenting a novel paracrine mechanism by which BFR resistance training mediates muscle growth. In addition, the coexisting increase in muscle protein synthesis with satellite cell activity [122] may lend some support to the notion that there is a synergism between autocrine and paracrine mechanisms that ultimately contributes to the hypertrophic adaptations of BFR resistance training.

7 Conclusions

A growing body of research has demonstrated the robust hypertrophic effects of resistance training with BFR, which can produce positive training adaptations at intensities

lower than previously believed (<50 % 1RM). Although the use of BFR resistance exercise is indeed intriguing and effective, the mechanisms underpinning the hypertrophic adaptations are yet to be fully determined. It has been suggested that increased levels of metabolic stress is the primary driving stimulus in this process, which is theorised to activate a number of other mechanisms (i.e. systemic hormone production, increased fast-twitch fibre recruitment), all of which are thought to mediate muscle growth via autocrine and/or paracrine actions. However, the extent to which these mechanisms are activated with metabolic stress is unclear. In fact, previous research suggests that some of these mechanisms are more mediated by mechanical tension (another primary impetus of muscle growth) rather than metabolic stress, which perhaps questions their level of contribution in BFR resistance training-induced hypertrophy given its low-intensity nature.

Despite the low level of mechanical tension associated with BFR resistance training, both mechanical tension and metabolic stress are primary factors of muscle hypertrophy, so it seems reasonable to conclude that both of these would synergistically contribute to the hypertrophic adaptations of BFR resistance training, with metabolic stress playing the dominant role. Both factors may mediate muscle hypertrophy through a combination of mechanisms as outlined above, all of which are thought to stimulate muscle protein synthesis by modulating signalling pathways in favour of muscle hypertrophy and/or increase satellite cell activation and proliferation. However, specific identification of the mechanisms most associated with the primary factors, as well as the particular extent of activation of each of the mechanisms by the primary factors requires further investigation. A complication with attributing causal description is that mechanical tension and metabolic stress occur in tandem, making it difficult to determine the relative involvement of each of them. This can potentially result in misinterpretation of the mechanisms thought to be associated with metabolic stress when in fact they are more mediated by mechanical tension, or vice versa.

A better understanding of the above mechanisms will lead to the development of optimal training programmes that maximise morphological adaptations. These approaches can then be applied in many clinical, rehabilitation, and athletic settings.

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