ACUTE RESPONSES TO EXERCISE WITH BLOOD FLOW RESTRICTION: A SYSTEMATIC REVIEW

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SIGNATURE PAGE

PROJECT: ACUTE RESPONSES TO EXERCISE WITH BLOOD FLOW RESTRICTION: A SYSTEMATIC REVIEW

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ABSTRACT

Exercise with blood flow restriction (BFR) has emerged as a promising method for augmenting muscle growth during low-intensity exercise programs which are otherwise not associated with substantial muscle hypertrophy, thus holding high efficacy in various at-risk populations. However, BFR programs are certainly not optimized, a fault of methodological disparities and a lack of mechanistic underpinnings. The purpose of this systematic review is to better elucidate these mechanisms and to understand the degree of cellular stress conjured by BFR exercise, and to use these results to provide further practical guidance for BFR exercise programming. Web of Science, PubMed, CINAHL, ScienceDirect, and PEDro were searched from January 1st 2010 to December 31st 2020 resulting in 1582 articles, of which 47 were included. Results showed that stress responses (e.g., muscle damage, oxidative and metabolic stress) are highly contingent on the exact exercise protocol and that inflammation in lieu of muscle damage appears to be a key orchestrator of muscle growth after exercise with BFR. Collective findings point to careful implementation of BFR exercise in untrained populations, such as avoiding resistance exercise to repetition failure, as exaggerated stress responses appear most pronounced in this population which would be counterproductive from an exercise adaptation standpoint.
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CHAPTER 1:
INTRODUCTION

Exercise with blood flow restriction (BFR) has emerged as an alternative to traditional high-load (HL) resistance exercise (RE) or aerobic exercise (AE) in both clinical and non-clinical populations. Exercise with BFR involves the use of a pneumatic pressure cuff placed around the proximal portion of a limb, such as the inguinal crease for the lower extremities (Laurentino et al., 2008) or just distal to the deltoid, above the bicep (Luebbers et al., 2014) for the upper extremities. The goal of BFR is to locally occlude venous return while restricting arterial blood flow to and from musculature, respectively, resulting in exacerbated metabolic stress from metabolite accumulation and hypoxia (Loenneke, Fahs, et al., 2013). Although the role of metabolic stress in skeletal muscle growth remains equivocal, an accreting body of evidence bolsters the efficacy of BFR as its application produces similar hypertrophic adaptations as HL resistance training (RT) while using lower loads that normally are not canonical to hypertrophy (Centner, Wiegel, et al., 2019; Lixandrão et al., 2018). Several general guidelines exist for designing RT programs with the goal of developing muscular strength and size, which include using loads equivalent to an individual’s 1-12 repetition maximum, corresponding to approximately \( \geq 65\% \) one-repetition maximum (1RM) (American College of Sports Medicine, 2009). Low-load RE with BFR (LL-BFR RE) and low-intensity AE (LIAE) drastically deviate from these directives as training with BFR applies much lower loads and higher repetitions than traditional HL-RT, typically ranging from 20-30\% 1RM for 15-30 repetitions each set (Pope et al., 2013). Exercise with BFR has been cogently represented as an effective alternative for those who are unable to exercise at higher
intensities due to vulnerabilities to high mechanical tension—including various clinical populations such as post-operative patients, older adults with underlying musculoskeletal complications, and those with osteoarthritis—by sufficiently increasing muscle strength and size (Grønfeldt et al., 2020; Lixandrão et al., 2018) with minimal risks and less pain than HL-RT (Cristina-Oliveira et al., 2020; Minniti et al., 2019). Aside from the morphological and neuromuscular adaptations observed pursuant to LL-BFR RT programs, the lack of pain experienced by those performing LL-BFR RT programs potentially results in increased adherence, which is relevant to clinical practice.

Whereas LL-RT is able to induce increases in muscular size similar to HL-RT, the use of such low-loads is suggested to require high training volumes and performing sets to momentary muscular failure (Lasevicius et al., 2019; Schoenfeld et al., 2017). The use of high volumes may be contraindicated in certain populations due to the level of perceived discomfort that may inhibit adherence to an exercise program. On the contrary, the addition of BFR to LL-RT has been suggested to elicit similar increases in muscle size as HL-RT with lower training volume than HL-RT and LL-RT, and perhaps without requiring training to failure (Farup et al., 2015; Kubo et al., 2006; Martín-Hernández et al., 2013; Vechin et al., 2015). This deviation between LL-RT with and without BFR supports the use of BFR in populations that may not be able to tolerate HL-RT or LL-RT to failure.

**Statement of the Problem**

Although BFR-RT has exhibited noticeable efficacy in various populations (Luebbers et al., 2014; Minniti et al., 2019; Pignanelli et al., 2020), the underlying biological mechanisms behind skeletal muscle morphological adaptation have not been
completely elucidated (Heitkamp, 2015; Hwang & Willoughby, 2019; Pearson & Hussain, 2015; Pope et al., 2013). Notably, it is unclear whether LL-BFR RE results in muscle damage, an acute inflammatory response, and an oxidative stress response. Currently, conclusions from research are difficult to interpret due to differences in methodology that, while nuanced, have definite influence on results and impact chronic adaptation.

**Purpose Statement**

The aim of this project will be to construct a systematic review manuscript focused on the acute responses to blood flow restriction exercise which include, muscle damage, inflammation, oxidative stress, pain, and fatigue.

**Significance**

The conclusions drawn from the present review may further elucidate the degree of stress that ensues exercise with BFR and provide valuable information to practitioners on the ideal frequency, type, intensity, volume, and other methods of implementation to further guide LL-BFR RT programming to produce optimal increases in hypertrophy and strength without accumulating significant tissue damage or provoking negative side effects (such as soreness). Such undesirable outcomes may reduce patient compliance and inhibit rehabilitative goals, counterintuitive to the use of BFR. Furthermore, evaluation of the oxidative stress response to LL-BFR RE is valuable as exercise-induced increases in oxidative stress not only accompanies skeletal muscle regeneration, but also may promote angiogenesis, mitochondrial biogenesis, hypertrophy (Gomes et al., 2012; Kosmidou et al., 2002; Vezzoli et al., 2011). Insight into the specific exercise stimuli provoking oxidative stress is therefore a contributor to programming to promote holistic adaptations.
Operational Definitions

Resistance Exercise – Resistance exercise (RE) is herein defined as a single session of exercise consisting of muscular contraction when subjected to an external resistance.

Resistance Training – Resistance training (RT) is described as repeated bouts of RE over a period of time.

Abbreviations

Akt – Protein Kinase B

AMPK – Adenosine Monophosphate Kinase

CAT – Catalase

CK – Creatine Kinase

DGC – Dystrophin-Associated Glycoprotein Complex

eEF2 – Eukaryotic Translation Elongation Factor 2

ERK1/2 – Extracellular Signal-Regulated Protein Kinase 1/2

bFGF – Basic Fibroblast Growth Factor

FOXO – Forkhead Box O

GASP-1 – G-protein Coupled Receptor-Associated Sorting Protein 1

GSH – Glutathione

HIF-1α – Hypoxia-Inducible Factor 1-alpha

HSP27 – Heat Shock Protein 27

IGF-1 – Insulin-like Growth Factor 1

IL-10 – Interleukin 10

IL-6 – Interleukin 6

LDH – Lactate Dehydrogenase

MAPK – Mitogen-Activated Protein Kinase
MARPs – Muscle Ankyrin Repeat Proteins
MCP-1 – Monocyte Chemoattractant Protein-1
MGF – Mechano-Growth Factor
miRNA – microRNA
MRF – Myogenic Regulatory Factors
MSTN – Myostatin
mTORC1 – Mechanistic Target of Rapamycin Complex 1
MuRF-1 – Muscle Ring Finger 1
Myf5 – Myogenic Factor 5
MyoD – Myoblast Determination Protein 1
NADH – Nicotinamide Adenine Dinucleotide
NF-κB – Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NO – Nitric Oxide
p70S6K / S6K1 – Ribosomal Protein S6 Kinase beta-1
PCr – Creatine Phosphate
PA – Phosphatidic Acid
PC – Protein Carbonyl
PI3K – Phosphoinositide 3-Kinase
SMAD – Mothers Against Decapentaplegic Homolog
SOD – Superoxide Dismutase
STAT1/3 – Signal Transducer and Activator of Transcription 1/3
SYDN4 – Syndecan-4
UA – Uric Acid
TAC – Total Antioxidant Capacity
TBARS – Thiobarbituric Acid-Reactive Substance
TGF – Transforming Growth Factor
TNF-α – Tumor Necrosis Factor-alpha
VASH1 – Vasohibin-1
VEGF – Vascular Endothelial Growth Factor
XO – Xanthine Oxidase
CHAPTER 2:
LITERATURE REVIEW

Introduction

Blood flow restriction (BFR) alone or combined with aerobic or resistance exercise (RE) has been utilized across various populations (Barili et al., 2018; Clark et al., 2011; Hughes, Patterson, et al., 2019; Karabulut et al., 2013) to improve muscular strength, size, and physical function in lieu of high mechanical loads commonly associated with these outcomes. This training method involves the use of pneumatic pressure cuffs wrapped around the proximal region of the upper or lower limbs inflated to a pressure intended to induce partial vascular occlusion, thus limiting arterial inflow of blood and occluding venous outflow (Patterson et al., 2019). The pressure used to obtain this effect has previously included absolute pressures, percentages of systolic blood pressure (BP), and percentages of arterial occlusion pressure [(AOP); i.e., the pressure required to completely cease blood flow to a limb]. Various exercise modalities, including walking, cycling, and low-load RE (LL-RE), are then conducted. This mechanical pressure in conjunction with physical exercise induces a hypoxic environment in the region of the limb distal to the cuff placement, conceivably augmenting metabolic stress endured by the contracting muscle tissue (Lauver et al., 2019; Yanagisawa & Fukutani, 2018) which is postulated to account for the hypertrophic response. This training method offers the advantage of less physical and perceived stress than present with high-intensity aerobic or RE (Bryk et al., 2016; Hughes, Patterson, et al., 2019; Hughes, Rosenblatt, et al., 2019), which has clear clinical application.
Due to the clinical relevance of exercise with BFR as a therapeutic intervention in sarcopenic and/or otherwise musculoskeletal compromised individuals, the overall safety of the method is necessary to consider. The nature of BFR implementation externally compresses vasculature, posing a potential threat to vascular integrity. Additionally, the hemodynamic response to exercise with BFR is important to consider with veneration to those with cardiovascular (CV) ailments as an augmented response could be contraindicated in an at-risk population. Exercise with BFR offers greatest efficacy in those who are unable to tolerate resistance training (RT) programs with high external loads, such as older adults. Considering endothelial dysfunction is a key component of the aging process (Ungvari et al., 2018) and the adoption of regular physical exercise appears to attenuate this process (Rossman et al., 2017), it is pivotal that older adults perform regular physical exercise. Specifically, it appears that this improvement in endothelial function supervenes both aerobic exercise and RE, with effects dependent upon frequency rather than intensity of RE (Ashor et al., 2015). This is an important distinction as LL-RE with BFR (LL-BFR RE) can potentially be performed more frequently than high-load RE (HL-RE) without the same potential to accumulate tissue damage (Nielsen et al., 2012; Nielsen et al., 2017) and/or result in non-functional overreaching/overtraining syndrome. Additionally, traditional aerobic exercise alone is not typically associated with an increase in muscle size, which is a necessary consideration as aging is coordinated with muscle atrophy and changes in muscle morphology (e.g., replacement with fat and connective tissue) (Lexell, 1995; Lexell et al., 1988). On the contrary, low-intensity aerobic exercise combined with BFR may provide the necessary CV stimulus to induce positive changes in endothelial function, and the
The hypertrophic benefit of BFR fosters the blunting of sarcopenia. Furthermore, muscular function and health are enhanced in resistance trained and aerobically trained individuals compared to their untrained counterparts (Aagaard et al., 2007; Bathgate et al., 2018), substantiating the rationale for continued aerobic exercise and RE in the aging population. Therefore, it is necessary to assess potential safety risks of BFR prior to implementation as a high risk/reward relationship would contraindicate use in older adults.

Under traditional HL-RT, high external load is thought to elicit a myofibrillar protein synthetic response via mechanotransduction, a process potentially consisting of a myriad of mechanically-sensitive molecular events including signaling via PA, DGC, the α7β1 integrin complex, the focal adhesion vinculin–talin–integrin complex, desmin, and MARPs (Bamman et al., 2018). In response unaccustomed physical activity—related to variables such as mechanical tension, volume, duration, muscle contraction type, muscle length, and intensity—microtrauma to skeletal muscle fibers, known as exercise-induced muscle damage (EIMD), may occur. Subsequently, four phases of skeletal muscle regeneration take place: necrosis, inflammation, repair, and scar-tissue formation (Ambrosio et al., 2009). Therefore, the ensuing immune response is a pivotal component of the rejuvenation process and can be presumed an indicator of microtrauma. Within the BFR literature, however, conflicting and incomplete data exists on the branching physiological mechanisms between LL-BFR RE and hypertrophic outcomes. Currently, questions remain regarding the mechanism driving the hypertrophic adaptive response provided by a properly incorporated LL-BFR RT program. Specifically, it is unclear whether LL-BFR RT results in EIMD and a concomitant local acute inflammatory
response, which are potential—albeit debated—regulators of muscle hypertrophy (Schoenfeld, 2012; Wackerhage et al., 2019). Previous research exhibits equivocal data on EIMD, which—as subsequently discussed—is likely attributed to methodological differences in variables including load intensity, cuff pressures, and/or contraction type. Furthermore, discrepancies may also result from dissimilar set and repetition schemes across studies, with some utilizing a frequently implemented regimen for LL-BFR RE of one set of 30 repetitions and three sets of 15 repetitions while other studies have instructed participants to perform exercise to muscular failure. Additionally, the oxidative stress response to LL-BFR RE—another potential yet dubious regulator of muscle hypertrophy (Pearson & Hussain, 2015; Schoenfeld, 2013)—is unclear, primarily due to methodological discrepancies between studies and the difficulty in isolating metabolic stress from other potential regulators of muscle growth (Garten et al., 2015; Goldfarb et al., 2008; Neto et al., 2018; Takarada, Nakamura, et al., 2000). Taken together, the data on EIMD, the immune response, and oxidative stress following LL-BFR RE is dearth, leaving much to uncertainty.

The growing popularity of exercise with BFR in both clinical and research settings requires both an improved understanding of the mechanisms behind the observed adaptations and a continual refinement of the methods of implementation to optimize rehabilitative programming and increase study homogeneity. Therefore, the purpose of the present review is to evaluate the safety and clinical relevance of exercise with BFR, to discuss the mechanisms behind neuromuscular (NM) and skeletal muscle morphological adaptations to exercise with BFR, and finally to bring forth current recommendations for implementation of BFR.
Safety of Blood Flow Restriction

Considering BFR manipulates and disrupts normal hemodynamics, the potential stress on the CV system and thereby, overall safety have been called into question and appropriately evaluated in prior reviews of literature (Cristina-Oliveira et al., 2020; Loenneke, Wilson, et al., 2011). The effects of blood pooling and subsequent alternation of post-exercise blood flow during exercise with BFR have been examined as this phenomenon poses potential danger to vascular integrity. Previous research has found an overall increase in the CV response—including heart rate (HR), BP, and cardiac output (Q)—during aerobic exercise with BFR versus without in both healthy young and hypertensive older adult populations (Barili et al., 2018; Renzi et al., 2010; Shimizu et al., 2016; Thomas et al., 2018). Additionally, Renzi et al. (2010) found decreased local vasodilation, an outcome contrary to normal CV responses to exercise, after a 14-minute walking protocol with BFR compared to a non-BFR protocol, which may represent a threat to vascular health. This blunting of normal endothelial function is corroborated by research indicating exercise with BFR results in a lack of increase in flow-mediated vasodilation acutely (Paiva et al., 2016; Tinken et al., 2009; Tinken et al., 2010) and a decrease in flow-mediated vasodilation after 4 weeks of training (Credeur et al., 2010). However, this chronic outcome is not ubiquitously supported as improvements in vascular endothelial function after 4 weeks have also been reported (Shimizu et al., 2016). Nonetheless, these findings propose a greater degree of stress on the CV system and an abnormal endothelial response during exercise using BFR compared to without, warranting caution prior to BFR use in those with CV disease. On the contrary to an augmentation in CV stress, May, Brandner, & Warmington (2017) assessed CV
responses to different intensities of aerobic exercise (running at 80% VO\(_2\)max, and walking at 4 km·h\(^{-1}\) with and without BFR) and overall found a similar increase in the hemodynamic response between low intensity aerobic exercise with and without BFR, which was lower than high intensity exercise without BFR. In response to resistance exercise (RE), May et al. (2017) found that hemodynamic variables increased to a greater extent during LL-BFR RE than without, and certain variables (diastolic BP and mean arterial pressure) increased to a greater extent than HL-RE as well. Similarly, Poton & Polito (2015) reported elevated hemodynamic variables in LL-BFR RE compared to without, though no increase in the hemodynamic response over HL-RE was observed. Additionally, Curty et al. (2018) found that supramaximal eccentric RE [130% one-repetition maximum (1RM)] with and without BFR similarly increased the CV response. However, this may merely reflect a maximal CV response to the supramaximal load that is physiologically unable to further increase by the addition of BFR, curtailing valuable extrapolation of these findings. These differences between CV responses to aerobic and resistance exercise with and without BFR likely result from dissimilar methods of implementing BFR cuff pressure, making collective results enigmatic. Holistically, research suggests that CV workload is elevated during low-intensity aerobic and RE with BFR compared to without, albeit still lower than high-intensity exercise. Though this consensus may be dependent upon BFR cuff pressure implementation, the apparent increase in acute stress and the abnormal endothelial response should be considered by practitioners before executing BFR programs with patients with CV disease. The CV responses to RE with and without BFR are generalized along with clinical outcomes in table 1. Based on the aforementioned observations, future research assessing the CV
responses to BFR should adopt current suggested methods of BFR implementation—via (Patterson et al., 2019)—to promote data comparison.

Contrary to concern for negative effects of exercise with BFR on vascular integrity and the CV system, the method may instead provide beneficial adaptations. One such adaptation may be enhanced post-exercise blood flow (Patterson & Ferguson, 2010), possibly due to capillary stress-induced angiogenesis (Evans et al., 2010; Hudlicka & Brown, 2009). In agreement with this conclusion, Ferguson et al. (2018) reported greater mRNA expression of VEGF, a signaling protein involved in angiogenesis (Shibuya, 2011), after a single bout of LL-BFR RE compared to without. Additionally, an increase in circulating miRNA associated with angiogenesis has recently been reported following a single bout of LL-BFR RE (Vogel et al., 2019). However, this conclusion is disputable when studying long-term changes. Following a 6-week training period, Pignanelli et al. (2020) found similar increases in capillarization between LL-RE with and without BFR. This seemingly contradictory evidence may be explained by the observation that acute modifications in gene expression are not necessarily manifested as chronic physiological adaptations. These acute changes do, however, indicate the presence of vascular stress, potentially from an ischemia/reperfusion induced increase in reactive oxygen species (ROS) production and oxidative stress (Barili et al., 2018; Patterson et al., 2019), which is a known stimulant of molecular angiogenic pathways (Kim & Byzova, 2014). Furthermore, the lack of a notable increase in capillarization found by Pignanelli et al. (2020) may not be indicative of an absence of stress, but may rather be attributed to an increased expression of VASH1, which is speculated to both increases endothelial cell tolerance to oxidative stress and inhibits angiogenesis (Sato, 2015). However, this notion
has not previously been envisaged or examined in the context of BFR. Nonetheless, assessment of arterial function indicates that no changes in peripheral arterial resistance after 4 weeks of exercise with BFR occur: an additional testament to safety (Clark et al., 2011). However, data was collected on young, healthy males, which could be viewed as a substantial limitation to generalizability.

Another potential concern following a BFR program is thrombus formation. Studies evaluating the risk of venous thrombus formation following a training program using BFR have collectively found no increased risk in healthy younger and older populations (Clark et al., 2011; Fry et al., 2010; Laswati et al., 2018; Madarame et al., 2010). While rare, cases of rhabdomyolysis have been reported following exercise with BFR (Iversen & Røstad, 2010; Tabata et al., 2016). This risk warrants caution prior to prescribing BFR in certain clinical patients. One way to monitor potential negative effects of a BFR exercise program is to maintain consistent measurements of muscle damage, including CK and various interleukins (Lee et al., 2017) to ensure no excessive muscle damage is occurring. This has been practically implicated as Karabulut, Sherk, Bemben, and Bemben (2013) evaluated levels of CK and IL-6 during 6 weeks of LL-BFR RT in older adults. Analysis of data found no increases in either of these markers, indicating a lack of chronic, elevated muscle damage and inflammation. Generally, the risk of adverse effects from BFR is rare; reports have indicated the incidence of venous thrombus formation to be <0.06% and rhabdomyolysis to be <0.01% (Nakajima et al., 2006; Patterson & Brandner, 2018). Considering BFR is most useful as a temporary intervention for various clinical patients to preserve or improve skeletal muscle mass before progressing to higher training intensities, proposed long-term risks are likely
curtailed. Therefore, evaluations of short-term use of BFR likely suffices in representing the comprehensive safety of implementing BFR to exercise programs. Use of BFR should be tailored to the individual, dependent upon the presence or absence of a diseased state.

**Clinical Use of Blood Flow Restriction**

Blood flow restriction has been successfully implemented in various clinical populations, including patients at risk of knee osteoarthritis (OA), sarcopenic individuals, and patients with ligament injuries. Commonly used resistance exercises in those at risk for knee OA include leg press and knee extensions (Bryk et al., 2016; Ferraz et al., 2018; Segal, Davis, et al., 2015; Segal, Williams, et al., 2015). Bryk et al. (2016) performed a 6-week exercise intervention in women diagnosed with knee OA to conduct a between-group comparison of HL-RT (70% 1RM) vs. LL-BFR RT (30% 1RM) on strength and knee pain. The authors reported no significant differences in changes in strength between groups, while participants experienced less knee pain during LL-BFR RT than the HL, no BFR group. Similarly, after a 12-week RT intervention in woman aged 50-65 years, Ferraz et al. (2018) reported comparable increases in muscular strength and quadriceps size between HL-no BFR and LL-BFR RT, with LL-RT without BFR eliciting inferior outcomes. The increase in muscle strength and size from LL-BFR RT accompanied a decrease in pain from pre- to post-intervention, which was not observed during HL-RT (no BFR). Furthermore, A 4-week intervention by Segal, Davis, et al. (2015) assessed the effectiveness of BFR in men aged ≥45 years at risk of knee OA by comparing LL-RT with and without BFR. Results of this study conflict with the notion that the addition of BFR augments changes in muscle strength as participants of both interventions exhibited similar increases in leg press 1RM. However, the time course of this study should be
considered a potential limitation as increasing the duration may have resulted in differences between groups. Importantly, neither group reported exacerbation of knee pain, indicating training with BFR is not inferior to training without BFR when considering perceived pain. In a similar 4-week study, the efficacy of BFR was appraised in women at risk for knee OA (Segal, Williams, et al., 2015). Contrary to Segal, Davis, et al. (2015), Segal, Williams et al. (2015) found a significantly greater improvement in muscular strength with LL-BFR RT vs. without. In agreement with other research, participants did not experience an increase in knee pain in either group. This decrease in perceived knee pain during exercise has definite clinical application as it can promote compliance and therefore enhance rehabilitative and/or quality of life outcomes.

Dissonant strength outcome measures between Segal, Davis, et al. (2015) and Segal, Williams, et al. (2015) may be reflective of differences in the history of regular physical activity of the participants as neural adaptations maintain the predominant mechanism behind initial improvements in strength (Gabriel et al., 2006). These are clear examples of the clinical relevance of LL-BFR RT.

In older adults, previously studied exercise interventions include various upper and lower body resistance exercises (Libardi et al., 2015; Shimizu et al., 2016; Vechin et al., 2015; Yasuda et al., 2015) as well as unloaded walking (Abe et al., 2010; Clarkson et al., 2017; Iida et al., 2011; Ozaki et al., 2011). Low-load RT with BFR is a well substantiated intervention method as it has generally resulted in greater increases in size and strength (Libardi et al., 2015; Vechin et al., 2015; Yasuda et al., 2015) when compared to LL-RT without BFR. Blood flow restriction has shown to be widely applicable—and thus possesses invaluable use to various clinical situations—as unloaded
walking with BFR also resulted in an increase thigh muscle size and strength and physical function above that observed with unloaded walking without BFR (Abe et al., 2010; Clarkson et al., 2017; Iida et al., 2011; Ozaki et al., 2011). While both muscle size and strength generally exhibit a significantly greater increase during LL-BFR RT vs. without, strength does not appear to increase to the same magnitude as with HL-RT without BFR (Cook et al., 2017; Vechin et al., 2015).

Further evidence supports the potential benefits of BFR during post-orthopedic surgery rehabilitation, though one study suggests that BFR implementation is not able to successfully preserve skeletal muscle mass as the attenuation of muscle atrophy was not found to be different between LL-BFR RT and LL-RT without BFR after 2 weeks of exercise intervention (Iversen et al., 2016). However, several studies demonstrate the value of BFR during post-operative rehabilitation for patients who are unable to perform HL-RT due to pain and/or compromised tissue integrity (Hughes, Paton, et al., 2018; Hughes, Patterson, et al., 2019; Hughes, Rosenblatt, et al., 2019; Ladlow et al., 2018). In support, after an 8-week intervention 2-weeks post-surgery, Hughes, Rosenblatt, et al. (2019) reported no condition interaction for changes in muscle morphology (thickness and pennation angle), strength (10RM), and knee joint laxity between LL-BFR RT and HL-RT. Additionally, LL-BFR RT improved knee function to a greater degree and exhibited lower perceived pain compared to HL-RT, which is consistent with other prior reports (Hughes, Paton, et al., 2018; Hughes, Patterson, et al., 2019). Ladlow et al. (2018) corroborates these findings as male subjects with lower-body injury exhibited similar improvements in muscular strength and size as well as greater improvements in functional tests with LL-BFR RT vs. HL-RT. Furthermore, pursuant to 14-weeks of RT,
Centner, Laubet, et al. (2019) found similar increases in Achilles tendon cross-sectional area (CSA) and stiffness between HL-RT and LL-BFR RT. This finding is intriguing considering that it is canonical that chronic, high loading is responsible for such adaptations (Bohm et al., 2015; Bohm et al., 2014; Wiesinger et al., 2015). This observation has led to postulation that the hypoxic environment created by BFR is responsible for the aforementioned adaptations. However, this hypothesis is based on speculation from studies that indicate hypoxia stimulates the proliferation of tendon stem cells (Jiang et al., 2014) and augments the secretion of bFGF (Shill et al., 2017), both of which ultimately lead to collagen synthesis and tendon repair. Regardless of mechanism, the body of evidence (summarized in table 1) presents LL-BFR RT as an effective post-surgery rehabilitative approach to increase musculotendinous size and strength and enhancing function comparable to HL-RT and superior to LL-RT.
Table 1. Comparison of low-load resistance exercise / resistance training with BFR to high-load and low-load without BFR on training outcomes.

<table>
<thead>
<tr>
<th>Outcome Variable</th>
<th>LL-BFR vs HL</th>
<th>LL-BFR vs Without</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>↓</td>
<td>↔</td>
</tr>
<tr>
<td>Muscle Size</td>
<td>↔</td>
<td>↑ or ↔</td>
</tr>
<tr>
<td>Muscular Strength</td>
<td>↓</td>
<td>↑ or ↔</td>
</tr>
<tr>
<td>CV Stress</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Endothelial Function</td>
<td>-</td>
<td>↓ or ↑</td>
</tr>
</tbody>
</table>

LL-BFR = Low-load resistance training with blood flow restriction, HL= High load resistance training, ↓ = decreased/lower in LL-BFR, ↔ = no difference, ↑ = increased/greater in LL-BFR

Neuromuscular Aspects of Blood Flow Restriction Resistance Exercise

According to Henneman’s size principle, larger motor units innervating type II muscle fibers are recruited as greater muscular force is required to meet the external resistance of higher training loads (Henneman et al., 1965). Under normal physiological circumstances, motor units—individually defined as an α motor neuron and the muscle fibers it innervates—are recruited in a sequential, additive manner. Both α motor neurons and motor units vary in size: smaller α motor neurons innervating relatively few low-force producing muscle fibers (type I) are typically recruited to contract against relatively light loads while larger α motor neurons innervating a relatively large number of high-force producing muscle fibers (type II) are additionally recruited as external load increases (Purves et al., 2018). As such, smaller motor units have a lower recruitment threshold than larger motor units, aligning with the phenotypic characteristics and function of their respective innervated muscle fibers. However, LL-BFR RE potentially allows for early recruitment of type II fibers even at low external loads (Loenneke, Abe, et al., 2012). It is postulated that hypoxia and metabolite accumulation result in higher threshold type II motor unit recruitment to indemnify the reduced force development due to inadequate oxygen supply to type I fibers which are recruited first (Dankel et al., 2017;
Loenneke, Abe, et al., 2012; Schoenfeld, 2013). Furthermore, hypoxia and metabolic stress may also result in the stimulation of group III and IV afferent fibers which act at spinal and supraspinal levels to inhibit α motor neurons and thus efferent signals responsible for muscle contractions (Laurin et al., 2015). This inhibition of muscle fiber excitation-contraction leads to a decrease in force production, which has been hypothesized to explain the increased recruitment of type II motor units to maintain necessary force output (Loenneke, Fahs, et al., 2011; Yasuda et al., 2010). Several studies have demonstrated an augmentation of type II fiber recruitment during BFR-RE using electromyography (EMG) (Fatela et al., 2019; Lauver et al., 2019; Loenneke, Fahs, et al., 2011; Yasuda et al., 2009) and through split P_i peak (Suga et al., 2012). Additionally, work by Lauver et al. (2017) and Manini et al. (2011) revealed a greater increase in muscle activation during LL-RE with BFR vs. without. In opposition, some studies have demonstrated similar muscle activation during LL-RE with and without BFR (Buckner et al., 2019; Freitas et al., 2020; Kacin & Strazar, 2011; Wernbom et al., 2009). These discrepancies may be explained by methodological differences as muscle activity variance depends upon the presence muscular fatigue (i.e., training to momentary muscular failure) and contraction type (i.e., concentric vs eccentric) (Duchateau et al., 2006; Enoka & Duchateau, 2008). Furthermore, Dankel et al. (2018) investigated the effects of BFR in addition to HL-RE (70% 1RM), and found no increases in muscle activity compared to HL-RE without BFR. These findings may be explained by the upper limit of motor unit recruitment (Duchateau et al., 2006), signifying that HL-RE is likely a sufficient stimulus to meet this limit, negating the effects of adding BFR at higher external loads. Additionally, LL-BFR RE does not necessarily recruit as many type II
fibers or elicit similar increase in muscle activity as HL-RE, signifying that mechanical tension has a greater role in recruitment (Cook et al., 2013; Freitas et al., 2020; Jessee et al., 2019; Manini & Clark, 2009; Suga et al., 2009). It should also be considered that the recruitment of type II fibers and strength adaptation have been intimated to require higher AOP values of ~80% (Lixandrão et al., 2015; Patterson et al., 2017; Suga et al., 2012), which is disadvantageous as this presents additional risks and potentially greater discomfort that may deter individuals from complying to a program using this method of training.

Data on both central and peripheral NM adaptations are also conflicting. Brandner, Warmington, & Kidgell (2015) reported an acute increase in corticomotor excitability in untrained participants following LL-BFR RE that was greater than that from HL-RE, indicating a possible benefit for patients who require increased motor function. Considering participants were untrained, this research is useful in presenting that changes in acute neurological function do occur with BFR in lieu of heavy external loads that typically permit this response. The differences in chronic adaptations between LL-BFR RT and HL-RT are not abundantly clear. Clark et al. (2011) reported no changes in nerve conduction velocity after 4 weeks of LL-BFR RT or HL-RT, though both increased isotonic strength. Cook, Scott, Hayes, & Murphy (2018) reported corroborative results as neither moderate-load nor LL-BFR RT exhibited changes in central or peripheral activation following 6 weeks of training. Additionally, following 12 BFR exercise sessions over 4 weeks, Colomer-Poveda, Romero-Arenas, Vera-Ibáñez, Viñuela-García, & Márquez (2017) reported no alterations in neurophysiological measurements despite an increase in muscle thickness, suggesting no changes in motor neuron
excitability and neural drive. These findings are consistent with the notion that much of the increases in strength following BFR-RT programs are resultant of myofiber hypertrophy rather than neural adaptations. Regarding overall muscular activation, a 12-week intervention by Kubo et al. (2006) resulted in a significant increase in EMG amplitude in HL-RT and no changes in LL-BFR RT, while a 16-week intervention by Takarada, Takazawa, et al. (2000) resulted in similar EMG amplitudes between LL-BFR RT and HL-RT. Cogently, the data on both acute long-term muscle activity is conflicting.

Regardless of neural adaptations, an increase in strength is seemingly apparent from LL-BFR RT. After 12 weeks of training 3 days per week, Kubo et al. (2006) reported a significant increase in maximum voluntary contraction (MVC) from both LL-BFR RT and HL-RT that were not statistically different. Following a 6-week RT protocol, Karabulut et al. (2010) found significant increases from baseline in leg press and leg extension strength for both LL-BFR RT and HL-RT. Interestingly, increases in leg press strength were not statistically different between groups for the former exercise, yet increases in strength in HL-RT were significantly greater than LL-BFR RT for the latter exercise, further obscuring collective findings. After training twice per week for 8 weeks, Laurentino et al. (2012) found no significant differences between increases in strength in LL-RT (20% 1RM), LL-BFR RT (20% 1RM), and HL-RT (80% 1RM). The authors noted, however, that the delta change in 1RM values in the LL group (20.7%) was significantly lower than that of the LL-BFR and HL groups (36.2% and 40.1%, respectively). A 5-week training study by Martín-Hernández et al. (2013) also found a significant increase in 1RM performance following LL-BFR RT in weight-training naïve males, although the improvements in strength were greater after HL-RT. A study by
Pignanelli et al. (2020) utilized a within-subject unilateral design in untrained male participants and found similar increase in strength after 6 weeks of training in BFR and non-BFR legs. While this study did not contain a HL group for comparison between HL and LL-BFR training, the results show that LL-RE with and without BFR produced similar strength gains, indicating a lack of superiority of either method in this case. In support of the notion that strength increases similarly between LL-BFR RT and HL-RT, Ellefsen et al. (2015) utilized a protocol in which LL-BFR RT was allocated to one leg while HL-RT was allocated to the other in untrained female participants. After 12 weeks of training, both protocols were found to be equally effective in increasing strength. However, it should be recognized that the LL-BFR RT leg was subjected to a much greater weekly volume-load (sets × reps × load) than the HL-RT leg. In tandem, the increase in strength resultant of LL-BFR RT programs, taken as equally effective as HL training or not, should be considered valuable as this provides definite clinical relevance.

Overall, it has been suggested that the observable increase in strength effectuated from LL-BFR RT can be attributed to muscle hypertrophy rather than NM adaptations commonly seen in HL-RT, indicated by a delayed increase in strength from LL-BFR RT programs when compared to HL-RT programs (Loenneke, Wilson, et al., 2012; Vechin et al., 2015). While the effects of LL-BFR RT on strength are debatable—with reviews of evidence intimating it produces similar strength gains to HL-RT (Grønfeldt et al., 2020) and other reviews of evidence indicating LL-BFR RT is not as effective as HL-RT (Centner, Wiegel, et al., 2019; Lixandrão et al., 2018)—it is a useful tool for those who are unable to tolerate RT with heavier loads, such as patients rehabilitating injuries, injury prone older adults, and those with compromised musculoskeletal systems.
Blood Flow Restriction and Skeletal Muscle Growth

It is traditionally accepted that the increased mechanical tension exerted on muscle resulting from heavy resistance exercise is the primary driving factor of muscle growth through stimulation of myofibrillar protein synthesis (Damas et al., 2016; Goldberg et al., 1975; Miller et al., 2005; Pearson & Hussain, 2015; Phillips et al., 2012; Spangenberg et al., 2008; Vandenburgh, 1987; Vandenburgh & Kaufman, 1979).

However, the role of metabolic stress as a potent stimulus for muscle hypertrophy has been discussed extensively (Schoenfeld, 2013; Wackerhage et al., 2019) and LL-BFR RE represents a significant metabolic stressor as it results in a greater degree of metabolite accumulation, oxygen extraction, and hypoxia than LL-RE without BFR (Ganesan et al., 2015; Lauver et al., 2017; Lauver et al., 2019; Yanagisawa & Fukutani, 2018; Yanagisawa & Sanomura, 2017). The greater metabolic stress resulting from LL-BFR RE is further substantiated by evidence of an increased level of blood lactate and acetyl-CoA carboxylase phosphorylation (a downstream target of AMPK) when compared to LL-RE without BFR (Nyakayiru et al., 2019). This marks LL-BFR RE as a cogent target for promoting hypertrophy in the absence of high mechanical tension, which is apparent as unloaded walking with BFR has previously been shown to increase muscle mass (Abe et al., 2006; Abe et al., 2010; Ozaki et al., 2011; Sakamaki et al., 2011). Furthermore, various studies on the effects of LL-BFR RT consistently find a similar increase in muscle size when compared with traditional, HL-RT (Biazon et al., 2019; Cook et al., 2018; Ellefsen et al., 2015; Kubo et al., 2006; Laurentino et al., 2012; Martín-Hernández et al., 2013). In support of the role of metabolic stress in hypertrophy, Biazon et al. (2019) reported a correlation between changes in deoxyhemoglobin and changes in
muscle CSA during LL-BFR RT, and no additional hypertrophic benefit from the addition of BFR to HL-RT. These findings further support that LL-BFR RT induced metabolic stress is adequate in producing a similar increase in muscle size as HL-RT and also suggests a redundancy of HL-RT with BFR, which is an important note for clinical application. Low-load RT with BFR may also promote appreciable muscle hypertrophy in a shorter time period (i.e., 3-6 weeks) than typically observed with HL-RT (Abe et al., 2006; Hill et al., 2018; Nielsen et al., 2012; Takada et al., 2012), and potentially requires lower training volume than LL-RE without BFR (Farup et al., 2015), marking it as an invaluable tool for clinical application.

The exact mechanisms connecting LL-BFR RE and the accompanying metabolic stress to skeletal muscle hypertrophy have yet to be fully understood, but have been postulated to involve various cell signaling cascades such as stimulation of the PI3K/Akt/mTORC1/p70S6K and MAPK/ERK1/2 pathways, and the inhibition of eEF2 kinase, the myostatin SMAD2/3 pathway, and dephosphorylation of FoxO transcription factors (Ge & Chen, 2012; Loenneke, Abe, et al., 2012; Mammucari et al., 2007; Ozaki et al., 2014; Schiaffino et al., 2013; Schoenfeld, 2013; Stitt et al., 2004; Tzivion et al., 2011; Wernbom et al., 2013). These pathways ultimately alter gene expression to favor myofibrillar protein synthesis over protein degradation, resulting in an accretion of myofibrillar proteins and eventual hypertrophy of the muscle fibers. Empirically, LL-BFR RE has resulted in an increase in phosphorylation of mTORC1 and S6K1, along with decreased phosphorylation of eEF2, resulting in a significant increase in myofibrillar protein synthesis compared to LL-RE without BFR (Fry et al., 2010; Fujita et al., 2007). Furthermore, Laurentino et al. (2012) reported a similar decrease in MSTN mRNA
expression in LL-BFR RT and HL-RT, with no changes in non-BFR LL-RT. Additionally, the expression of GASP-1 and SMAD-7, genes associated with inhibition of MSTN function, increased in both LL-BFR RT and HL-RT but not in LL-RT without BFR. This research also reported similar increase in muscle CSA in HL-RT (6.1% increase) and LL-BFR RT (6.3% increase), while LL-RT without BFR did not experience statistically significant changes. Additionally, Manini et al. (2011) found a downregulation in the expression of proteolytic genes (namely, MuRF-1, Atrogin-1, and FOXO3A) in all participants who underwent LL-BFR RE while LL-RE without BFR showed no changes. However, alterations in myogenic transcripts were not observed in either group. Finally, Ellefsen et al. (2015) found no differences in changes in gene expression between LL-BFR RT and HL-RT, indicating a similar response to each protocol; though it should be considered that LL-RT without BFR was not included, limiting the interpretation of these findings. The potential mechanism behind the increase in muscle size due to LL-BFR RT based on these collective findings is represented in figure 1. In tandem, the cell biological evidence indicates similar changes in signaling between LL-BFR RE and traditional HL-RE. Notably, it is important to consider the oscillatory nature of gene expression (i.e., rapid changes in upregulation and downregulation) in response to various environmental factors (including exercise) as this may explain differences between studies. Furthermore, differences in observed gene expression do not necessarily indicate inadequacies of training protocols or differences due to training intervention, but rather are a reflection of dynamic regulatory mechanisms.
Studies have also examined the changes in myonuclear content, fiber composition, and fiber size following LL-BFR RT. An increase in myonuclei and activation of dormant satellite cells is necessary for regulating muscle fiber size since skeletal muscle nuclei are post-mitotic and thus, rely on the addition of nuclei from satellite cells to accommodate hypertrophy and promote cellular repair following injury (Murach et al., 2018). It has been insinuated that EIMD is an essential regulator of the muscle growth and repair process through stimulation of cytokine production and the immune response, which then direct satellite cells to the damaged area (Quinn, 2008; Serrano et al., 2008; Tidball & Villalta, 2010). However, there is still a lack of certainty whether LL-BFR RE results in significant muscle damage, with previous reviews of evidence suggesting that EIMD does not occur (Allsopp & May, 2017; Loenneke, Thiebaud, & Abe, 2014). Despite a lack EIMD to elicit myonuclear genesis and increase satellite cell activity, these outcomes have been documented following LL-BFR RT. Some evidence in these outcome measures have reported an increase in muscle fiber size,
number of myonuclei, and number of satellite cells in both type I and type II fibers (Bjørnsen, Wernbom, Løvstad, et al., 2019; Nielsen et al., 2012). On the contrary, a recent study by Pignanelli et al. (2020) reported no changes in fiber type, fiber CSA, myonuclei, or myonuclear domain following 6-weeks of LL-RT with or without BFR. Similarly, Ellefsen et al. (2015) found no changes in myonuclei per muscle fiber following 12 weeks of LL-BFR RT or HL-RT. These findings may be attributed to an overall lower training frequency in the latter studies compared to the former. Nonetheless, a novel finding by Ellefsen et al. (2015) was the increased expression of SYND4 after HL-RT and LL-BFR RT. The SYND4 gene demonstrably has a role in satellite-cell mediated muscle regeneration (Cornelison et al., 2001; Cornelison et al., 2004), evidently in response to EIMD (Casar et al., 2004). Though Ellsefsen et al. (2015) did not assess muscle damage, acceptance of the premise that EIMD does not result from LL-BFR RE combined with evidence of increased expression of SYND4 favors the notion that satellite cell activity increases following LL-BFR RE in lieu of muscle damage.
**Figure 2.** Potential mechanism by which LL-BFR RE-induced hypoxia and metabolic stress result in an acute inflammatory response that can describe the observed increase in muscle size without an increase in muscle damage. (see Operational Definitions for abbreviated terms)

The acute immune response and ensuing satellite cell activity proceeding LL-BFR RE have been suggested to result from local hypoxia, leading to the expression of HIF-1α, which is associated with the increased levels of IL-6, IL-10, MCP-1, and TNF-α, possibly through NF-κB upregulation (Larkin et al., 2012; Leire et al., 2013; Liu et al., 2017; Natsume et al., 2019; Shill et al., 2017). In response to EIMD—or perhaps mechanical tension itself—these cytokines, as well as local macrophage and neutrophil accumulation, are requisite for muscle repair and hypertrophy through phagocytosis, satellite cell proliferation, stimulation of ERK1/2 and STAT1/3 pathways and MRFs (e.g., MyoD, Myf5, myogenin), and the eventual phosphorylation of mTORC1 through the release of IGF-1, TGF, bFGF, and MGF (Begue et al., 2013; Koh & Pizza, 2009; Rossi et al., 2018; Schoenfeld et al., 2016; Zanou & Gailly, 2013) (figure 2). Following LL-BFR RT, Nielsen et al. (2017) reported an increase in M1 and M2 macrophages and HSP27 without indicators of muscle damage; namely, CK. However, the authors declared
that macrophage accumulation was moderate compared to previous research reporting on the inflammatory response to muscle-damaging exercise. Furthermore, the increase in macrophage content occurred without commensurate increases in circulating cytokines—specifically, MCP-1, IL-6, and TNF-α—that are typically coupled with macrophage activation and polarization (Della Gatta et al., 2014; Tidball, 2011), indicating a possible lack of a regenerative response. This may further indicate a lack of muscle damage, thus precluding the need for muscle rejuvenation. After a single session of LL-BFR RE, Neto et al. (2018) also reported a lack of significant increase in CK and LDH, which supports the position that LL-BFR RE does not promote muscle damage. Conversely, Behringer, Heinke, Leyendecker, & Mester (2018) found similar increase in serum CK and neutrophil counts after HL-RE (75% 1RM) with and without BFR. This difference in EIMD can possibly be explained by methodological differences as Behringer et al. (2018) used higher loads and eccentric contractions, which are ostensibly more inducive of EIMD than lower loads and concentric contractions (Douglas et al., 2017; Enoka, 1996; Hasenoehrl et al., 2017). After a single bout of LL-BFR RE to volitional failure, Wernbom, Paulsen, Nilsen, Hisdal, & Raastad (2012) found an increase in intracellular tetranectin, most pronounced in type I fibers, which the authors attributed to an increase in sarcolemmal permeability and myofiber damage. Cumming, Paulsen, Wernbom, Ugelstad, & Raastad (2014) corroborated these findings with report of preferential stress—indicated by an increase in translocation of HSP27 and αβ-crystallin to cytoskeletal structures—to type I fibers after LL-BFR RE performed to momentary muscular failure, yet no indication of myofibrillar disruption was observed. The outcomes of these studies were likely impacted by the choice to execute RE to
momentary muscular failure, whereas many studies cease exercise prior to this point. However, these findings are consistent with the notion that type I fibers are preferentially stressed during LL-BFR RE due to prolonged tension (via set duration) and an inhibition of type II fiber recruitment resultant of acute metabolic acidosis (i.e., an increase in intracellular [H⁺] and subsequent disruption of Ca²⁺ binding), which further increases tension on type I fibers as they necessarily maintain force output. With respect to chronic adaptation and practicality, the increase in type I fiber stress may be manifested as preferential hypertrophy of type I fibers (Bjørnsen, Wernbom, Kirketeig, et al., 2019; Bjørnsen, Wernbom, Løvstad, et al., 2019; Jakobsgaard et al., 2018). Regarding differences in outcomes due to training to momentary muscular failure, studies by Patterson, Leggate, Nimmo, & Ferguson (2013) and Takarada, Nakamura, et al. (2000) reported an increase in IL-6 levels following a LL-BFR RE session while Bugera, Duhamel, Peeler, & Cornish (2018) did not find similar results. These disparate outcomes are likely manifestations of methodological differences as the former studies instructed participants to train to failure while the latter did not. This nuance has a potential biochemical impact as IL-6 is upregulated in response to multiple stimuli, including myotrauma and alterations in cellular energy status (Hennigar et al., 2017). As such, the increased plasma concentration of IL-6 observed by Patterson et al. (2013) and Takarada, Nakamura, et al. (2000) and not by Bugera et al. (2018) is conceivably due to differing degrees of stress on cellular energetics associated with training to failure rather than muscle fiber damage. With veneration to functional testing of EIMD, studies from various authors (Copithorne & Rice, 2019; Sieljacks et al., 2016; Umbel et al., 2009; Wernbom, Paulsen, Nilsen, Hisdal, & Raastad, 2012) reported prolonged decrements in
force production following LL-BFR RE, feasibly signifying muscle damage. However, this outcome is not ubiquitously supported (Alvarez et al., 2020; Loenneke, Thiebaud, et al., 2013; Thiebaud et al., 2014; Thiebaud et al., 2013), further questioning the veracity of findings on EIMD after LL-BFR RE. Clearly, data regarding muscle damage and the immune response after LL-BFR RE remains equivocal, with discrepancies likely due to methodological differences regarding loading, repetition scheme, volume, and contraction type. To further expound, the data is conflicting perhaps due to different set and repetition schemes as training to failure likely innately promotes a greater degree of muscle damage than training prior to failure (Hasenoehrl et al., 2017; Pareja-Blanco et al., 2018).

Oxidative stress is an additional factor that potentially influences muscle hypertrophy through ROS production and cell signaling (Schoenfeld, 2013). Oxidative stress constitutes a condition of an imbalance between the production and accumulation of ROS and the capacity of biological antioxidant defense systems to detoxify the reactive products (Pizzino et al., 2017). Under pathological conditions, excessive ROS production can lead to damage to various biomolecules, in turn damaging cells and tissue. However, there is a pivotal distinction between pathological ROS production and that ensued by exercise, the latter of which has various beneficial rather than harmful roles; this discussion is beyond the scope of this review but has been appropriately deliberated elsewhere (Simioni et al., 2018). The overall role of ROS in muscle hypertrophy is suppositional, through postulation that ROS production enhances MAPK signaling and IGF-1 function, promoting hypertrophy (Handayaningsih et al., 2011; Kefaloyianni et al., 2006). Nonetheless, training with the intent of generating high metabolic stress may
enhance the production of ROS and subsequent hypertrophy-stimulatory pathways. However, it is currently suggested that high mechanical tension and muscle damage are the primary factors responsible for ROS production during and after RE (Pearson & Hussain, 2015; Schoenfeld, 2013), potentially due to the HL-RE mediated recruitment of type II muscle fibers, which may be more inducive of ROS production (Quindry et al., 2011). While exercise—more specifically, muscle contraction—alone is an accepted mode of augmenting intramuscular ROS production primarily via the mitochondrial electron transport chain, NADH-oxidase, and XO (Ji, 1999; Powers & Jackson, 2008; Powers et al., 2011), and bouts of hypoxia and/or ischemia/reperfusion are associated with an increase in ROS production (Clanton, 2007; Leurcharusmee et al., 2018; Zuo & Clanton, 2005; Zuo et al., 2013), data coalesces to suggest no significant increase in oxidative stress occurs in response to LL-BFR RE. Takarada, Nakamura, et al. (2000) found no difference in lipid peroxide between the LL-RE with and without BFR, indicating a lack of augmentation of oxidative stress from the addition of BFR to LL-RE. Similarly, Boeno et al. (2018) reported no differences in CAT and a decrease in SOD without changes in concentration of NO metabolites following LL-BFR RE, indicating a lack of an antioxidant response. Goldfarb et al. (2008) reported greater levels of oxidative stress markers following HL-RE (70% 1RM) without BFR than LL-BFR RE (30% 1RM). Additionally, a study by Garten, Goldfarb, Crabb, & Waller (2015) consisted of 6 different conditions (resting, resting with BFR, 30% 1RM to failure, 30% 1RM to failure with BFR, 70% 1RM to failure, 70% 1RM to failure with BFR) resulted in variations in the oxidative stress response. The authors reported a greater level of oxidative stress markers during HL-RE (70% 1RM) with BFR than without BFR. Intriguingly, LL-RE
(30% 1RM) without BFR and applying BFR at rest both resulted in an increase in oxidative stress markers, yet the combination of LL-RE and BFR reduced the levels of oxidative stress markers. A 1-week study conducted by Nielsen et al. (2017) evaluated changes in GSH and TAC in LL-BFR RE (20% 1RM) and HL-RE (70% 1RM). Low-load RE with BFR demonstrated a lack of significant changes from baseline values for either GSH or TAC during the 24 hours following 2 different exercise sessions while an increase in oxidative stress was observed after HL-RE. This data aligns with the consensus that the use of high external load is the primary RE stimulus for oxidative stress. These studies instructed participants to perform RE to momentary muscular failure, leaving the effects of performing a specific number of repetitions per set to uncertainty. Furthermore, the oxidative stress response to RE is difficult to evaluate since the response depends on a variety of factors including sex, fiber type composition, training status, and mode and intensity of exercise (Siciliano et al., 2020). Transparently, limited data exists on the oxidative stress response following RE with BFR, especially in untrained individuals, rationalizing further investigation.

**Blood Flow Restriction Resistance Training Programming**

As with any RT program, a RT program implementing BFR must be designed with respect to variables including muscle contraction type, exercise selection, intensity, volume, frequency, rest intervals, and repetition velocity (Kraemer & Ratamess, 2004). A balance of these key variables with proper variation over time (i.e., periodization) is required when designing an optimized training program. Resistance exercise with BFR presents additional training variables such as pneumatic cuff pressures and size, variation in cuff pressure based on position of exercise, and whether intermittent or continuous
blood flow restriction should be utilized. These must also be carefully considered to maximize outcomes and minimize risks when designing a LL-BFR RT program.

Current recommendations for LL-BFR RE implementation include performing 75 total repetitions per exercise with 20-40% 1RM, distributed through one set of 30 repetitions followed by three sets of 15 repetitions with 30 second to one minute of rest between sets (Patterson et al., 2019). While the implementation of low rest periods (i.e., 30 seconds) between sets is rationalized with the goal of maximizing metabolic stress (Kraemer & Ratamess, 2004; Krzysztofik et al., 2019), this may result in attenuated recovery of muscular force production capacity during subsequent sets and/or exercises (Kraemer & Ratamess, 2004; Yasuda et al., 2013). This may manifest as consistently lower mechanical tension over a training period, potentially limiting the magnitude of the hypertrophic response. Further, some studies have opted for training to failure (Cumming et al., 2017; Cumming et al., 2014; Ellefsen et al., 2015; Wernbom, Paulsen, Nilsen, Hisdal, & Raastad, 2012), aligning with research indicating LL-RT to failure results in similar increase in muscle size as HL-RT (Schoenfeld et al., 2017) and suggestion that training with BFR to failure promotes type II fiber recruitment (Farup et al., 2015; Wernbom et al., 2009). Though it has been suggested that consistently training to failure may not be necessary to elicit these similar gains in muscle size during LL-RT with (Kubo et al., 2006) or without (Sampson & Groeller, 2016) BFR, this claim is not ubiquitously supported (Lasevicius et al., 2019). As with any RT program, periodized progression is a key component to maximizing benefits, which has previously been completed in the form of increasing training volume and/or intensity over time (Ellefsen et al., 2015; Ferraz et al., 2018; Karabulut et al., 2010; Laurentino et al., 2012; Vechin et
It should be noted that while a progressive increase in training volume typically exhibits a dose-dependent relationship with muscle hypertrophy (Krzysztofik et al., 2019), this may not be the case with LL-BFR RT (Martín-Hernández et al., 2013). In a clinical setting, it is more so apposite to specify loading parameters within the range of 20-40% 1RM with respect to stage of rehabilitation and discomfort level; for instance, intensity could be gradually increased over time with patient progression to optimize outcomes effectuated by higher mechanical tension.

While LL-BFR RE is an increasingly popular technique, implementation is a conserved disparity amongst studies. Notably, pneumatic cuff pressure is a source of variability, making comparison and extrapolation of results from various studies inscrutable. It is currently suggested to individualize pressure (via AOP) and to use a range between 40-80% AOP (Patterson et al., 2019) with the rationale that individualization will negate the inter-individual variability of blood flow disruption—such as that ensued from differences in limb circumference or BP (Jessee et al., 2016; Loenneke et al., 2015; Sieljacks et al., 2018)—that occurs when using arbitrary absolute pressures or percentage of systolic BP (Clarkson et al., 2020). However, supplementary guidance is required to ensure that discomfort is minimized without compromising the efficacy of LL-BFR RT; this effect is evident as the use of higher relative pressures likely offers no greater acute or chronic benefit (Counts et al., 2016) and provokes greater discomfort, a greater CV response, and a greater decrease in exercise volume compared to lower relative pressures (Mattocks et al., 2017). This phenomenon therefore requires a balance between use of pressures high enough to elicit potentiated metabolic stress and (potentially) type II fiber recruitment, yet low enough to eschew excessive discomfort.
Therefore, it has been suggested that lower relative pressures be used in clinical populations where undue discomfort could discourage adherence, while higher relative pressures should be reserved for use in healthy or athletic populations to maximize the benefits ensued by LL-BFR RT (Clarkson et al., 2020).

An additional nuanced variable is pneumatic cuff width. Evidently, narrower cuffs require higher pressures to attain AOP than wider cuffs (Jessee et al., 2016; Loenneke, Fahs, et al., 2012; Sieljacks et al., 2018; Weatherholt et al., 2019). However, the magnitude of these effects can be allayed by using individualized AOP, evident as Mouser et al. (2018) found no differences in changes in blood flow with varying relative pressures between wide and narrow cuffs. It has also been found that wider cuffs result in greater CV stress (i.e., higher HR, BP, rate pressure product, and mean arterial pressure) than narrower cuffs when using absolute pressures (Rossow et al., 2012), providing additional support for using AOP. Importantly, it appears that no differences in NM adaptations following a LL-BFR RT program occur between narrow and wide cuffs (Laurentino et al., 2016). Therefore, the use of either wide or narrow cuffs is sufficient so long as AOP is used as the method of pressure application.

While it is widely accepted that the use of AOP is a superior method to percentage of systolic BP or arbitrary absolute values, one additional point of concern is the body orientation-dependent variation in AOP. While a dearth of data exists on the variation of AOP based on body orientation, it appears that AOP is higher when standing than when seated, which is higher than when supine (Hughes, Jeffries, et al., 2018; Sieljacks et al., 2018). This has important implications as, for instance, measurement of AOP in the seated or standing position while exercise is performed in the supine position
could result in an excessively high occlusive pressure which can cause a greater CV response (Rossow et al., 2012), increase various health risks and reduce the efficacy of LL-BFR RT (Loenneke, Thiebaud, Abe, et al., 2014), and increase level of discomfort (Jessee et al., 2017; Mattocks et al., 2017). Furthermore, excessive occlusion pressures induce a similar a degree of hypoxia as lower pressures, indicating higher pressures likely do not profitably increase metabolic stress (Kilgas et al., 2019). Alternatively, insufficiently low pressures may not induce a heightened level of hypoxia and metabolic stress, which may compromise chronic adaptions to LL-BFR RT. These findings underscore the importance of determining AOP in the exercising position. The summarized guidelines for the training variables discussed and rationale for these currently suggested guidelines are presented in table 2. Following these guidelines not only increases safety and efficacy of BFR application, but also promotes homogeneity of future research.
Table 2. A list of BFR resistance training variables, potential issues they present, and proposed solutions based on the current literature recommendations.

<table>
<thead>
<tr>
<th>Training Variable</th>
<th>Potential Issues</th>
<th>Proposed Solution</th>
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<tbody>
<tr>
<td><strong>Load</strong></td>
<td>Discomfort and augmented responses to higher loads.</td>
<td>Individualize load within the range of 20-40%1RM based on individual characteristics.</td>
</tr>
<tr>
<td><strong>Repetitions</strong></td>
<td>Discomfort and augmented response to training to failure.</td>
<td>Use a 4 × 30,15,15,15 scheme in those unable to tolerate training to muscular failure.</td>
</tr>
<tr>
<td><strong>Rest</strong></td>
<td>Low rest periods may cause a decrease in force output and compromise load stimulus. High rest period may decrease metabolic stress and compromise this stimulus.</td>
<td>Individualize based on population (athletic, clinical, healthy non-athletic).</td>
</tr>
<tr>
<td><strong>Volume</strong></td>
<td>Unclear use of high or low volume of training.</td>
<td>LL-BFR RT may require lower overall training volumes than LL-RT without BFR and HL-RT.</td>
</tr>
<tr>
<td><strong>Frequency</strong></td>
<td>Higher frequencies of HL-RE are not recommended as inadequate recovery between sessions may lead to nonfunctional overreaching or overtraining.</td>
<td>LL-BFR RT does not appear to confer significant muscle damage even at very high frequencies [e.g., 23 bouts in 3 weeks (Nielsen et al., 2012)], indicating a higher possible training frequency compared to HL-RT without engendering negative outcomes.</td>
</tr>
<tr>
<td><strong>Cuff Pressure</strong></td>
<td>Method of implementation alters physiological responses.</td>
<td>Use 40-80% AOP (Patterson et al., 2019) to account for differences in individual characteristics and to limit potential negative side effects.</td>
</tr>
<tr>
<td><strong>Cuff Width</strong></td>
<td>Different required occlusive pressures &amp; physiological responses based on cuff width.</td>
<td>Use AOP to negate the effects of cuff width on absolute occlusive pressures.</td>
</tr>
<tr>
<td><strong>Body Position</strong></td>
<td>Differences in occlusion pressure due to body position could reduce the efficacy of BFR during LL-RE.</td>
<td>Measure AOP in the position of exercise to curtail the effects of body position on degree of restriction.</td>
</tr>
<tr>
<td><strong>iBFR vs cBFR</strong></td>
<td>Differences in perception or physiological responses may affect the efficacy of BFR.</td>
<td>Current literature does not substantiate meaningful differences between methods, suggesting either method is valid.</td>
</tr>
</tbody>
</table>
1RM = one-repetition maximum, LL-BFR RT = low-load resistance training with blood flow restriction, HL-RE = high-load resistance exercise, HL-RT = high-load resistance training, AOP = arterial occlusion pressure, LL-RE = low-load resistance exercise, iBFR = intermittent blood flow restriction, cBFR = continuous blood flow restriction

An emerging topic of interest is the potential differences between performing LL-BFR RE with cBFR versus iBFR. One point of concern of iBFR regards the ability to generate similar increases in metabolic stress and muscle activity as cBFR. Specifically, if iBFR dampens the degree of metabolic stress and muscle activation compared to cBFR, this may alter chronic adaptations (i.e., the magnitude of hypertrophy) to LL-BFR RT. Although cBFR is the more frequently employed method for applying BFR, previous studies have found a greater increase in muscle size in iBFR compared to LL-RT without BFR (Kacin & Strazar, 2011) and a similar increase in muscle size as HL-RT (Biazon et al., 2019). Regarding strength outcomes, evaluation by Kacin & Strazar (2011) found no differences in strength increases between iBFR and LL-RT without BFR while Biazon et al. (2019) reported similar increases in strength between iBFR and HL-RT. These dissimilar findings likely exist due to the duration of the latter study compared to the former (10 weeks vs 4 weeks), coinciding with the notion that muscular strength does not appreciably increase until about 10 weeks of LL-BFR RT (Loenneke, Wilson, et al., 2012). Furthermore, the findings by Biazon et al. (2019) indicate that although iBFR may not elicit similar metabolic stress (Okita et al., 2019; Suga et al., 2012) or type II fiber recruitment (Suga et al., 2012) as cBFR, similar increases in muscle size compared to HL-RT likely occur. Additionally, these differences between iBFR and cBFR are not completely substantiated as similar increases in muscle activity (Freitas et al., 2020; Yasuda et al., 2013) and metabolic stress (Freitas et al., 2020; Neto et al., 2017) between these methods have been reported. The disagreement between studies is a likely result of
dissimilar set and repetition schemes, methods of quantifying metabolic stress, and implementation of cuff pressures. Interestingly, Yasuda et al. (2013) reported a delayed recovery of MVC during cBFR compared to iBFR, which was speculated to be resultant of a lack of PCr repletion due to inadequate oxygen supply (Yoshida & Watari, 1997). However, this effect could likely be nullified by allocating longer rest periods or by using lower cuff pressures (i.e., AOP rather than an absolute pressure) (Sugaya et al., 2011). A potential advantage of iBFR over cBFR may be a lower level of discomfort (Fitschen et al., 2014; Yasuda et al., 2013), though this claim is refutable (Freitas et al., 2019; Neto et al., 2017) with differences likely stemming from divergent cuff pressures, set and repetition schemes, and training status. Lastly, previous research indicates neither cBFR nor iBFR are able to promote muscle damage (Neto et al., 2018), a finding supportive of an absence of acute differences between the two methods. The body of evidence regarding cBFR versus iBFR is conflicting, requiring further investigation in line with current BFR standards.

Conclusions

It is transparent that research utilizing BFR requires standardization as the present evidence on the various topics discussed is consistently conflicting. While some conclusions can be accepted from current research, clinically relevant findings benefit from research converging to a consensus, which is not currently the case for the topics discussed. Current disparities in research findings are, however, useful in forming a more refined method for conducting LL-BFR RT. Notably, data regarding EIMD, inflammation, and oxidative stress demonstrates substantial scarcity. The enigmatic data on these variables is most likely resultant of variance in BFR implementation and
program intervention. For instance, the differences between training to failure vs training with the commonly used approach of 4 sets of 30-15-15-15 reps, using differing cuff pressures and methods for implementation of such pressures, duration of rest periods, intensity and mode of exercise (i.e., isokinetic vs isotonic, concentric vs eccentric), and measurement of different indicators of muscle damage, inflammation, and oxidative stress at various time points. For instance, CK exhibits high inter-individual variability and requires prolonged evaluation (i.e., 3-7 days post exercise) to better indicate muscle damage, and IL-6 may be upregulated by a variety of stimuli (Coratella et al., 2016; Lee et al., 2017; Morán-Navarro et al., 2017; Pareja-Blanco et al., 2019; Sieljacks et al., 2016). Therefore, additional markers of EIMD are requisite for more veracious findings. Additionally, studies assessing the effects of LL-BFR RE compared to HL-RE require an adequate washout period to control for any potential repeated-bout effect. While the magnitude and relevance of this effect is uncertain, it is an important variable to consider as a protective effect is conferred by HL-RE as well as repeated bouts of LL-RE (Hyldahl et al., 2017) and, potentially, from LL-BFR RE (Sieljacks et al., 2016). An adequate washout period is therefore advised to negate any protective effect against subsequent muscle damage and oxidative stress that would influence physiological responses and provide inaccurate results (Koch et al., 2014; Nikolaidis et al., 2007). Nonetheless, current literature appears to suggest that no EIMD or inflammation indicative of EIMD occurs following LL-BFR RE. Additionally, ubiquitous findings indicate LL-BFR RE is not capable of provoking appreciable ROS generation. Regarding practical application, it appears that programing and research should be predicated on alignment with the currently indicated BFR implementation methods to better establish homogeneity and
promote comparative analysis. Namely, implementation should, at minimum, incorporate the following: 1) measurement of AOP to determine pneumatic cuff pressure rather than absolute values or percentages of systolic BP, 2) measurement of AOP in the position of exercise to better promote comfortability and minimize variance due to cuff size while not deterring BFR-induced metabolic stress and 3) perform sets to volitional failure or for 4 sets of 30-15-15-15 reps at 20-40% 1RM (Patterson et al., 2019).
CHAPTER 3:  
METHODOLOGY

Literature Search Strategy

A computer assisted database search was conducted including Web of Science, PubMed, Cumulative Index of Nursing and Allied Health Literature (CINAHL), ScienceDirect, and Physiotherapy Evidence Database (PEDro) and search results included dates from January 1\textsuperscript{st} 2010 to December 31\textsuperscript{st} 2020. Search terms included “blood flow restriction”, “partial vascular occlusion”, “occlusion training”, “kaatsu”, “muscle damage”, “skeletal muscle damage”, “exercise-induced muscle damage”, “oxidative stress”, “reactive oxygen species”, “free radical”, “inflammation”, “genetic”, “hormone”, “hypertrophy”, “gene”, “satellite cell”, “epigenetic”, “miRNA”, “mRNA”, and “protein synthesis”. Additionally, previous germane reviews were examined for further identification of eligible references.

Inclusion and Exclusion Criteria

Inclusion criteria of the literature search were as follows: 1) studies written in English and published in peer-reviewed journals, 2) randomized-controlled trials, 3) at least one condition or group consisting of an exercise intervention or skeletal muscle electrical stimulation combined with BFR, 4) acute data comprising at least one indicator of muscle damage, inflammation, oxidative stress, an endocrine response, and/or gene expression. Papers were excluded if the study used means other than external mechanical compression (i.e., hyperbaric chamber or hypoxic environment rather than pneumatic pressure cuff or tourniquet) or if the study utilized non-human participants; furthermore, paper types excluded included reviews, systematic reviews, meta-analyses, abstracts, case
reports, supplements, reports, pilot studies, study protocols, and/or opinion articles. Reasons for exclusion are additionally included in the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) flowchart (figure 3).

Figure 3. PRISMA flowchart of study selection process.
**Study Selection**

All identified articles across all databases were saved using a citation manager (EndNote Online) and duplicates were subsequently removed. All remaining articles were screened for inclusion based on the title and abstract and, in the event of unclear study relevance, the full article was retrieved for further review. Successively, full-text documents of all relevant articles were retrieved and further excluded if fulfilling any of the exclusion criteria.

**Assessment of Study Quality**

Study quality was assessed utilizing methods previously implemented (Ma et al., 2020). The PEDro scale was used to evaluate methodological quality of trials (Maher et al., 2003). The PEDro scale consists of 11 “Yes/No” items, of which 10 (2-11) are scored. The unscored item (item 1 regarding eligibility criteria) is related to external validity and thus it is not used to calculate the PEDro score. Studies with scores 0-3 points are considered “low” quality, scores of 4-5 points “moderate” quality, and scores of 6-10 points “high” quality. The Center for Evidence Based Medicine’s Levels of Evidence (CEBM) scale (Table 3) was used as an auxiliary quality assessment of each eligible paper.
Table 3. Levels of Evidence adopted from Center for Evidence-Based Medicine, Oxford England.

<table>
<thead>
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<th>Types of Studies</th>
</tr>
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<tbody>
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<td>I</td>
<td>High-quality randomized controlled trial with statistically significant difference or no statistically significant difference but narrow confidence intervals&lt;br&gt;Systematic review of Level-I randomized controlled trials</td>
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</tr>
<tr>
<td>III</td>
<td>Case-control study&lt;br&gt;Retrospective comparative study&lt;br&gt;Systematic review of Level-III studies</td>
</tr>
<tr>
<td>IV</td>
<td>Case series</td>
</tr>
<tr>
<td>V</td>
<td>Expert opinion</td>
</tr>
</tbody>
</table>
REFERENCES


https://doi.org/10.1097/PHM.0b013e3181951fc5

https://doi.org/10.1249/MSS.0b013e3181915670


https://doi.org/10.1590/1677-5449.011017

https://doi.org/10.1186/s40798-015-0009-9


https://doi.org/10.1111/cpf.12557


Health Service trial. Phys Ther Sport, 39, 90-98.
https://doi.org/10.1016/j.ptsp.2019.06.014


aged tendon stem cell. Cell Biochem Biophys, 70(2), 967-973. https://doi.org/10.1007/s12013-014-0004-7


Single-Blind Randomized Controlled Trial. *Front Physiol*, 9, 1269.

https://doi.org/10.3389/fphys.2018.01269


https://doi.org/10.1249/MSS.0b013e3182625928


https://doi.org/10.1519/JSC.0000000000003454


https://doi.org/10.1249/MSS.0000000000000833

Laurentino, G. C., Ugrinowitsch, C., Roschel, H., Aoki, M. S., Soares, A. G., Neves, M.,
Exerc, 44*(3), 406-412. https://doi.org/10.1249/MSS.0b013e318233b4bc
Laurin, J., Pertici, V., Dousset, E., Marqueste, T., & Decherchi, P. (2015). Group III and
IV muscle afferents: role on central motor drive and clinical implications.
*Neuroscience, 290*, 543-551. https://doi.org/10.1016/j.neuroscience.2015.01.065
Lauver, J. D., Cayot, T. E., Rotarius, T., & Scheuermann, B. W. (2017). The effect of
eccentric exercise with blood flow restriction on neuromuscular activation,
microvascular oxygenation, and the repeated bout effect. *Eur J Appl Physiol,
117*(5), 1005-1015. https://doi.org/10.1007/s00421-017-3589-x
Neuromuscular and Microvascular Responses to Concentric and Eccentric
https://doi.org/10.1519/JSC.0000000000003372
Lee, E. C., Fragala, M. S., Kavouras, S. A., Queen, R. M., Pryor, J. L., & Casa, D. J.
(2017). Biomarkers in Sports and Exercise: Tracking Health, Performance, and
https://doi.org/10.1519/JSC.0000000000002122
inducible factor-1 in keratinocyte inflammatory response and neutrophil
28


https://doi.org/10.1007/s40279-017-0795-y


https://doi.org/10.1007/s00421-015-3253-2


https://doi.org/10.3389/fphys.2012.00392


Neto, G. R., Novaes, J. S., Salerno, V. P., Gonçalves, M. M., Batista, G. R., & Cirilo-Sousa, M. S. (2018). Does a resistance exercise session with continuous or intermittent blood flow restriction promote muscle damage and increase oxidative...

https://doi.org/10.1080/02640414.2017.1283430


https://doi.org/10.1249/mss.0b013e31804ca10c


https://doi.org/10.1007/s00421-012-2479-5


https://doi.org/10.1097/MCO.0b013e32834d19bc


https://doi.org/10.1152/ajpregu.00243.2019


https://doi.org/10.1155/2017/8416763


https://doi.org/10.1519/JSC.0b013e3182874721


https://doi.org/10.1519/JSC.0b013e31824f207e

https://doi.org/10.1007/s40279-013-0017-1

https://doi.org/10.1519/JSC.0000000000002200


https://doi.org/10.1177/2151458515583088

https://doi.org/10.1016/j.pmrj.2014.09.014


https://doi.org/10.1152/japplphysiol.90368.2008


Yanagisawa, O., & Fukutani, A. (2018). Effects of low-load resistance exercise with blood flow restriction on intramuscular hemodynamics, oxygenation level and

https://doi.org/10.23736/S0022-4707.17.07463-1


https://doi.org/10.1556/1646.9.2017.2.16


https://doi.org/10.1093/gerona/glut084


https://doi.org/10.1556/APhysiol.100.2013.4.6


PART 2:

SYSTEMATIC REVIEW MANUSCRIPT

The Acute Responses to Exercise with Blood Flow Restriction and Training

Implications: A Systematic Review

Daniel Helzer, MinHyuk Kwon, Michael Yi, and Edward Jo

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Abstract

Exercise with blood flow restriction (BFR) has emerged as a promising method for augmenting muscle growth during low-intensity exercise programs which are otherwise not associated with substantial muscle hypertrophy, thus holding high efficacy in various at-risk populations. However, BFR programs are certainly not optimized, a fault of methodological disparities and a lack of mechanistic underpinnings. The purpose of this systematic review is to better elucidate these mechanisms and to understand the degree of cellular stress conjured by BFR exercise, and to use these results to provide further practical guidance for BFR exercise programming. Web of Science, PubMed, CINAHL, ScienceDirect, and PEDro were searched from January 1st 2010 to December 31st 2020 resulting in 1582 articles, of which 47 were included. Results showed that stress responses (e.g., muscle damage, oxidative and metabolic stress) are highly contingent on the exact exercise protocol and that inflammation in lieu of muscle damage appears to be a key orchestrator of muscle growth after exercise with BFR. Collective findings point to careful implementation of BFR exercise in untrained populations, such as avoiding resistance exercise to repetition failure, as exaggerated stress responses appear most
pronounced in this population which would be counterproductive from an exercise adaptation standpoint.

**Introduction**

Blood flow restriction (BFR) alone or combined with aerobic exercise (AE) or resistance exercise (RE) has been utilized across various populations to improve muscular strength, size, and physical function in lieu of high mechanical loads commonly associated with these outcomes (Barili et al., 2018; Clark et al., 2011; Hughes, Patterson, et al., 2019; Karabulut et al., 2013). This training method involves the use of pneumatic pressure cuffs wrapped around the proximal region of the upper or lower limbs inflated to a pressure intended to induce partial vascular occlusion, thus limiting arterial inflow of blood to and occluding venous outflow from the exercising musculature (Patterson et al., 2019). The determination of cuff pressure parameters have previously been based on absolute pressures, percentages of systolic blood pressure, and percentages of arterial occlusion pressure [(AOP); i.e., the pressure required to completely cease blood flow to a limb]. Various exercise modalities, including walking, cycling, and low-load RE (LLRE), are then performed. This external mechanical pressure in conjunction with physical exercise induces a hypoxic environment in the region of the limb distal to the cuff placement, theoretically augmenting metabolic stress within the contracting muscle(s) (Lauver et al., 2019; Yanagisawa & Fukutani, 2018) which is postulated to enhance or elicit a hypertrophic response. This training method offers the advantage of less mechanical stress than that experienced with high-intensity AE (HIAE) or high-load RE (HLRE) but with similar hypertrophic outcomes to the latter (Bryk et al., 2016; Hughes,
Patterson, et al., 2019; Hughes, Rosenblatt, et al., 2019), which has major clinical implications.

Under traditional HLRE, high external load is thought to elicit a myofibrillar protein synthetic response via mechanotransduction, a process consisting of a myriad of mechanically-sensitive molecular events including signaling via phosphatidic acid, the dystrophin-glycoprotein complex, the α7β1 integrin complex, the focal adhesion vinculin–talin–integrin complex, desmin, and muscle ankyrin repeat proteins (Bamman et al., 2018). Additionally, in response to unaccustomed physical activity—related to variables such as mechanical tension, volume, duration, muscle contraction type, muscle length, and intensity—microtrauma to skeletal muscle fibers, known as exercise-induced muscle damage (EIMD), may occur. Subsequently, four phases of skeletal muscle regeneration take place: necrosis, inflammation, repair, and scar-tissue formation (Ambrosio et al., 2009). Therefore, the ensuing immune/inflammatory response is a pivotal component of the rejuvenation process and is generally presumed an indicator of microtrauma. While the hypertrophic effects of BFR are well established, the BFR literature exhibits conflicting and incomplete data regarding the branching physiological mechanisms between exercise with BFR and these noteworthy hypertrophic outcomes. Specifically, it is unclear whether LLRE with BFR (LL-BFR RE) results in EIMD and a concomitant local acute inflammatory response, which are potential—albeit contemplated—regulators of muscle hypertrophy (Schoenfeld, 2012; Wackerhage et al., 2019). Previous research cultivates equivocal data on EIMD, which—as subsequently discussed—is attributable to foremost methodological differences in variables including volume-load, cuff pressures, rest periods, mode of exercise, and/or contraction type. To
elaborate, discrepancies likely result from dissimilar set and repetition schemes across studies, with some utilizing a frequently implemented regimen for LL-BFR RE of 75 total repetitions disseminated though one set of 30 repetitions and three sets of 15 repetitions while other studies have instructed participants to perform exercise to muscular failure (Behringer et al., 2018; Bugera et al., 2018; Callanan et al., 2020; Cumming et al., 2017; Cumming et al., 2014; Ellefsen et al., 2015; Fry et al., 2010; Gundermann et al., 2012; Gundermann et al., 2014; Neto et al., 2018; Wernbom et al., 2012; Winchester et al., 2020). Along with ambivalent stress responses to exercise with BFR is the oxidative stress response—another potential yet dubious regulator of muscle hypertrophy (Pearson & Hussain, 2015; Schoenfeld, 2013). This is an intriguing predicament as a surge of reactive oxygen species (ROS) production occurs during somewhat analogous ischemic/reperfusion (Zhou et al., 2018). Aside from RE, low-intensity AE (LIAE) (e.g., walking, cycling) combined with BFR has previously shown to induce appreciable muscle hypertrophy (Abe et al., 2006; Abe et al., 2010; Ozaki et al., 2011; Sakamaki et al., 2011). This of course leads to a series of questions regarding the underlying mechanism of action as LIAE is not typically associated with muscle growth. Given that metabolic stress has been deemed a key regulator of muscle hypertrophy (Schoenfeld, 2013), a series of hypertrophy-related mechanisms necessitate discussion, and exercise with BFR provides a novel and prime model of metabolic stress.

The growing popularity of exercise with BFR in both clinical and research settings requires an improved understanding of the mechanisms behind the observed adaptations to allow for continual refinement of the methods of implementation and optimization of rehabilitative programming. This crucial information is also salient for
future research as increased study homogeneity would necessarily enhance the quality of evidence and applicability of findings. Therefore, the purpose of this review is to systematically evaluate the acute responses to exercise with BFR to explicate the current inscrutable mechanisms behind skeletal muscle morphological adaptations to exercise with BFR—including the EIMD, inflammatory, metabolic stress, oxidative stress, and intracellular hypertrophic responses—and to evaluate current methods of implementation to bring forth supplementary recommendations for BFR exercise programming.

**Methodology**

**Literature Search Strategy**

A computer assisted database search was conducted including Web of Science, PubMed, Cumulative Index of Nursing and Allied Health Literature (CINAHL), ScienceDirect, and Physiotherapy Evidence Database (PEDro) and search results included dates from January 1st 2010 to December 31st 2020. Search terms included “blood flow restriction”, “partial vascular occlusion”, “occlusion training”, “kaatsu”, “muscle damage”, “skeletal muscle damage”, “exercise-induced muscle damage”, “oxidative stress”, “reactive oxygen species”, “free radical”, “inflammation”, “genetic”, “hormone”, “hypertrophy”, “gene”, “satellite cell”, “epigenetic”, “miRNA”, “mRNA”, and “protein synthesis”. Additionally, previous germane reviews were examined for further identification of eligible references.

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Figure 1. PRISMA flowchart of study selection process.

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Study quality was assessed utilizing methods previously implemented (Ma et al., 2020). The PEDro scale was used to evaluate methodological quality of trials (Maher et al., 2003). The PEDro scale consists of 11 “Yes/No” items, of which 10 (2-11) are scored. The unscored item (item 1 regarding eligibility criteria) is related to external validity and thus it is not used to calculate the PEDro score. Studies with scores 0-3 points are considered “low” quality, scores of 4-5 points “moderate” quality, and scores of 6-10 points “high” quality. The Center for Evidence Based Medicine’s Levels of Evidence (CEBM) scale (Table 1) was used as an auxiliary quality assessment of each eligible paper.

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| II    | Lesser-quality randomized controlled trial (e.g., <80% follow-up, no blinding, or improper randomization)  
Prospective comparative study  
Systematic review of Level-II studies or Level-I studies with inconsistent results |
| III   | Case-control study  
Retrospective comparative study  
Systematic review of Level-III studies |
| IV    | Case series |
| V     | Expert opinion |

Results

Studies Included

The database search resulted in a total of 1582 potentially relevant papers (PubMed n = 303, Web of Science n = 449, ScienceDirect n = 653, CINAHL n = 129, PEDro n = 48). After duplicate removal, 815 papers remained and were individually
screened based on titles and abstracts; following which, 77 papers remained. After accessing full-texts and further exclusion, 47 papers were eligible for qualitative evaluation. Assessment of study quality using the PEDro scale resulted in twelve “high” quality studies and 35 “moderate” quality studies. Based on the CEBM level of evidence scale, 46 of the included studies were considered level II while only one was considered level I. The level I study (Christiansen et al., 2018) was the only paper to explicitly state a method of blinding, thus providing a score of 7/10 (PEDro) and level I ranking (CEBM). Appositely, no level III, IV, or V studies were included as per the established eligibility criteria for inclusion. Individual references along with their corresponding PEDro and CEBM scores are presented in Table 2. Additionally, a recapitulation of the included studies is presented in Table 3.

### Table 2. List of included references (n = 47) along with their PEDro and CEBM scores.

<table>
<thead>
<tr>
<th>Reference</th>
<th>PEDro Score (Quality)</th>
<th>CEBM Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aguayo et al. 2016</td>
<td>5/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Amani-Shalamzari et al 2019</td>
<td>6/10 (high)</td>
<td>II</td>
</tr>
<tr>
<td>Amani-Shalamzari et al 2020</td>
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<td>II</td>
</tr>
<tr>
<td>Barili et al. 2018</td>
<td>6/10 (high)</td>
<td>II</td>
</tr>
<tr>
<td>Barjaste et al. 2020</td>
<td>6/10 (high)</td>
<td>II</td>
</tr>
<tr>
<td>Behringer et al. 2017</td>
<td>6/10 (high)</td>
<td>II</td>
</tr>
<tr>
<td>Behringer &amp; et al. 2018</td>
<td>5/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Boeno et al. 2018</td>
<td>5/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Bugera et al. 2018</td>
<td>4/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Cai et al. 2018</td>
<td>4/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Callanan et al. 2020</td>
<td>5/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Centner et al. 2018</td>
<td>6/10 (high)</td>
<td>II</td>
</tr>
<tr>
<td>Centner et al. 2019</td>
<td>4/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Christiansen et al. 2018</td>
<td>7/10 (high)</td>
<td>I</td>
</tr>
<tr>
<td>Conceicao et al. 2016</td>
<td>5/10 (moderate)</td>
<td>II</td>
</tr>
<tr>
<td>Cook et al. 2014</td>
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<tr>
<td>Cumming et al. 2014</td>
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<tr>
<td>Domeles et al. 2016</td>
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<td>II</td>
</tr>
<tr>
<td>Dos Santos et al. 2020</td>
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<td>II</td>
</tr>
<tr>
<td>Ellefsen et al. 2015</td>
<td>5/10 (moderate)</td>
<td>II</td>
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Table 2. (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Score (Quality)</th>
<th>Design</th>
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<td>Winchester et al. 2020</td>
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Effects of BFR on Muscle Damage and Inflammation

Fourteen studies were selected for their outcome measures related to acute EIMD and inflammation (Behringer et al., 2017; Behringer et al., 2018; Callanan et al., 2020; Cumming et al., 2017; Cumming et al., 2014; Dorneles et al., 2016; Dos Santos et al., 2020; Neto et al., 2018; Nielsen et al., 2017; Penailillo et al., 2020; Shill et al., 2017; Sieljacks et al., 2016; Wernbom et al., 2012; Winchester et al., 2020). Extrapolated results are conflicting regarding the potential of exercise with BFR to result in muscle damage using paradigmatic markers [e.g., creatine kinase (CK), myoglobin], with five
(Behringer et al., 2018; Dorneles et al., 2016; Penailillo et al., 2020; Sieljacks et al., 2016; Winchester et al., 2020) indicating that LLRE and eccentric cycling with BFR are capable of inducing muscle damage while three (Dos Santos et al., 2020; Neto et al., 2018; Nielsen et al., 2017) appear to flout these findings. Data is further convoluted with the inclusion of aberrant markers of myocellular stress (e.g., heat-shock protein content and expression) as studies posit LL-BFR RE is able to induce similar perturbation to muscle fiber integrity as LLRE without BFR and HLRE (Cumming et al., 2017; Cumming et al., 2014; Wernbom et al., 2012). Contrarily, using fatty acid binding protein as a muscle damage marker, the application of BFR to AE has been suggested to confer negligible muscle damage (Behringer et al., 2017).

The inflammatory response to exercise with BFR was evaluated by five of the included studies (Callanan et al., 2020; Dorneles et al., 2016; Dos Santos et al., 2020; Nielsen et al., 2017; Shill et al., 2017). The cytokines listed here include those principally implicated in inflammation, thus excludes the multifaceted myokines [e.g., interleukin(IL)-6, IL-15] (Piccirillo, 2019) as these are subsequently mentioned (refer to “Hormonal and Myokine Response to BFR”). The cytokine response included a decrease in monocyte chemoattractant protein-1 (MCP-1) but no change in tumor necrosis factor alpha (TNF-α) 24 hours after LL-BFR RE (Nielsen et al., 2017), and an increase in IL-10 and TNF-α during handgrip exercise with BFR compared to non-BFR at a 30-min time point (Shill et al., 2017). The leukocyte response to LL-BFE RE exhibits greater discrepancy. Dos Santos et al. (2020) discovered leukocytosis and lymphocytosis immediately after LL-BFR RE with no further changes 24- and 48- hours post-exercise, and no changes in neutrophils or monocytes at any time point. Contrarily, Dorneles et al.
(2016) reported a decrease in C-C chemokine receptor type 5 (CCR5) cells 24-hours after LL-BFR RE. Lastly, Callanan et al. (2020) found an increase in cluster of differentiation (CD)34+, platelet count, leukocytes, and lymphocytes immediately after LL-BFR RE, a decrease in neutrophils immediately after 40-min, and 60-min post exercise, and a decrease in lymphocytes 60-min post exercise.

**BFR and Oxidative Stress**

Ten of the included studies presented data on oxidative stress and endogenous antioxidant systems (Barili et al., 2018; Boeno et al., 2018; Centner et al., 2019; Centner et al., 2018; Christiansen et al., 2018; Garten et al., 2015; Item et al., 2013; Neto et al., 2018; Nielsen et al., 2017; Petrick et al., 2019). In response to LL-BFR RE and whole body vibration (WBV) with BFR, six studies (Boeno et al., 2018; Centner et al., 2019; Garten et al., 2015; Neto et al., 2018; Nielsen et al., 2017; Petrick et al., 2019) tendentiously presented a lack oxidative stress ensued by either mode of exercise. Nonetheless, (Centner et al., 2018) reported an increase in systemic ROS production following LL-BFR RE, providing contradictory evidence. Combination of HLRE with BFR putatively augments the oxidative stress response (Garten et al., 2015), an outcome that is also evident with the addition of WBV (Item et al., 2013). Additionally, Barili et al. (2018) provided evidence of oxidative stress in response to LIAE with BFR, specifically in older hypertensive females; and Christiansen et al. (2018) reported an increase in heat shock protein (HSP)27 protein content, as well as catalase and HSP70 mRNA expression, signifying oxidative stress consequential of LIAE with BFR in young, recreationally active males.

**Hormonal and Myokine Response to BFR**
Studies measuring hormonal and myokine responses were abundant and encompassed 23 of the eligible papers (Amani-Shalamzari et al., 2019; Amani-Shalamzari et al., 2020; Barjaste et al., 2020; Behringer et al., 2017; Behringer et al., 2018; Bugera et al., 2018; Cai et al., 2018; Conceicao et al., 2016; Cook et al., 2014; Ellefsen et al., 2015; Fry et al., 2010; Inagaki et al., 2011; Kim et al., 2014; Kraemer et al., 2016; Layne et al., 2017; Manini et al., 2011; Manini et al., 2012; Nielsen et al., 2017; Ozaki et al., 2014; Ozaki et al., 2017; Patterson et al., 2013; Shill et al., 2017; Winchester et al., 2020).

Regarding the versatile myokine IL-6, (Shill et al., 2017) reported an increase during handgrip exercise with BFR. This increase in IL-6 also appears to occur in response to HLRE and LLRE irrespective of BFR (Patterson et al., 2013; Winchester et al., 2020). Yet, Bugera et al. (2018) found no detectable levels of circulating IL-6 in response to LL-BFR RE. Comparably, no changes in plasma IL-6, IL-6 protein abundance, or IL-6 mRNA were found pursuant to LLRE or LIAE with BFR (Conceicao et al., 2016; Fry et al., 2010; Nielsen et al., 2017). Other measured myokines included IL-15, decorin, and irisin, of which decorin and irisin have been shown to increase in response to LL-BFR RE (Bugera et al., 2018; Kraemer et al., 2016). The muscle growth-inhibiting myokine myostatin has been shown to be unaltered in response to AE and LLRE with and without BFR though measurements of both serum levels and myostatin mRNA (Amani-Shalamzari et al., 2019; Conceicao et al., 2016; Manini et al., 2011).

Growth factors exhibit divergent responses to exercise with BFR. For instance, growth hormone (GH) has been shown to increase similarly following HLRE and differing intensities of AE regardless of BFR (Behringer et al., 2017; Behringer et al.,
increase to a greater extent in LL-BFR RE vs HLRE (Manini et al., 2012), increase in response AE, WBV, electromyostimulation (EMS), and LLRE with BFR to a greater extent than without (Amani-Shalamzari et al., 2020; Barjaste et al., 2020; Cai et al., 2018; Fry et al., 2010; Inagaki et al., 2011; Patterson et al., 2013), and increase similarly between HLRE and LL-BFR RE (Ellefsen et al., 2015; Kim et al., 2014). Insulin-like growth factor-1 (IGF-1) data displays slightly more convergence with a holistic lack of change following HLRE, AE, and LLRE with or without BFR (Amani-Shalamzari et al., 2019; Behringer et al., 2018; Conceicao et al., 2016; Manini et al., 2011; Manini et al., 2012; Ozaki et al., 2014; Patterson et al., 2013), while one study found similar increases following AE with and without BFR (Behringer et al., 2017), and another showed a greater increase from AE with BFR versus without (Barjaste et al., 2020). Layne et al. (2017) analyzed hepatocyte growth factor (HGF) and reported lower values following LL-BFR RE compared to without BFR in untrained participants. Lastly, Shill et al. (2017) found an increase in basic fibroblast growth factor during handgrip exercise with BFR above without.

The data on testosterone and cortisol is also conflicting, with studies indicating deviating responses including: a similar testosterone increase after WBV and AE with and without BFR (Behringer et al., 2017; Cai et al., 2018), greater testosterone levels after AE with BFR compared to non-BFR (Amani-Shalamzari et al., 2020), a greater increase in testosterone following HLRE with BFR versus without (Cook et al., 2014), and a lack of change in testosterone levels after AE regardless of BFR (Ozaki et al., 2014). Comparably dissonant, the reported cortisol responses include similar changes between BFR and non-BFR AE (Amani-Shalamzari et al., 2020; Behringer et al., 2017),
BFR and non-BFR EMS (Inagaki et al., 2011), and HLRE compared to LL-BFR RE (Kim et al., 2014); other research has found an increase in cortisol after LL-BFR RE only (Ellefsen et al., 2015), and even a larger increase during HLRE, AE and, LLRE with BFR compared to without (Cook et al., 2014; Fry et al., 2010; Ozaki et al., 2017). Conversely, cortisol has been reportedly unaffected following AE with BFR, without BFR, and LL-BFR RE (Barjaste et al., 2020; Ozaki et al., 2014; Patterson et al., 2013). Two studies also included measurements of norepinephrine and found greater values after AE with BFR compared to without (Ozaki et al., 2017) and no difference between EMS with and without BFR (Inagaki et al., 2011). Lastly, insulin has been found to increase following AE with BFR (Ozaki et al., 2014), and to a greater extent with BFR versus without (Ozaki et al., 2017).

**BFR Induced Metabolic Stress**

Several studies quantify metabolic stress through blood lactate concentration, while others opted for molecular and metabolomic approaches to analyze the established gene expression and metabolite responses associated with endurance exercise-related adaptations. Regarding blood lactate, eight of the included papers coalesce with the finding that lactate concentration is greater with the addition of BFR to LLRE, WBV, and AE compared to their non-BFR counterparts (Amani-Shalamzari et al., 2020; Cai et al., 2018; Callanan et al., 2020; Centner et al., 2019; Fry et al., 2010; Gundermann et al., 2012; Ozaki et al., 2017; Penailillo et al., 2020). This is corroborated by evidence of lower blood pH during running with BFR compared to without (Christiansen et al., 2018). Moreover, evidence provided by Dos Santos et al. (2020) indicates LL-BFR RE is capable of provoking an equal increase in lactate as HLRE, though this finding is not
undisputed (Centner et al., 2018; Kim et al., 2014; Manini et al., 2012; Valerio et al., 2018). Behringer et al. (2018) compared HLRE with and without BFR and found the addition of BFR to HLRE did not result in different lactate levels. Additionally, Behringer et al. (2017) and Barjaste et al. (2020) have found similar lactate between BFR and non-BFR sprints, and no change in blood lactate after BFR walking, respectively. Additionally, Christiansen et al. (2018) reported no change in PCr, Cr, or PCr/Cr following running bouts with BFR.

Given the reduction of blood flow and reputed decrease in oxygen delivery and availability to muscle fibers, several studies have evaluated the changes in hypoxia-inducible factor 1-alpha (HIF-1α). The expression of HIF-1α has been found to increase following LIAE with BFR (Barjaste et al., 2020; Conceicao et al., 2016), but remain unchanged after a single bout of LL-BFR RE (Fry et al., 2010) or HLRE with BFR and WBV performed to failure (Item et al., 2013).

Other molecular markers of metabolic stress (i.e., those associated with endurance exercise adaptations) include adenosine monophosphate-activated protein kinase (AMPK) and its downstream targets acetyl-CoA carboxylase (ACC) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α). It has been previously established that phosphorylation of AMPK at threonine 172 is critical for its enzymatic activity (Willows et al., 2017). Thus, four of the included studies evaluated AMPK phosphorylation and protein abundance. In response to higher- and lower-intensity AE with and without BFR, AMPK phosphorylation and abundance was reportedly unaffected (Christiansen et al., 2018; Conceicao et al., 2016). These findings corroborate results following LLRE with and without BFR (Fry et al., 2010; Wernbom et
On the contrary, ACC phosphorylation was found to increase after sprints with BFR (specifically, in type I fibers) (Christiansen et al., 2018) and to a greater degree pursuant to LL-BFR RE compared to non-BFR (Nyakayiru et al., 2019). The PGC1-α responses to exercise include an increase after BFR walking (Barjaste et al., 2020) and HIAE with BFR (Christiansen et al., 2018), a larger increase following HIAE compared to LIAE with BFR (Conceicao et al., 2016), a larger increase after HLRE with BFR and WBV compared to HLRE alone (Item et al., 2013), and a similar response between LL-BFR RE and HLRE (Ellefsen et al., 2015).

Endurance exercise is highly bioenergetically strenuous and is associated with changes in mitochondrial morphology, autophagy, and mitophagy, facilitating skeletal muscle adaptation and mitochondrial biogenesis (Fritzen et al., 2016; Jamart et al., 2012; Möller et al., 2015; Schwalm et al., 2015), providing impetus for analyzing markers of these responses to exercise with BFR. Regarding mitochondria, electron transport chain proteins have been found inert after LLRE and LIAE with BFR (Conceicao et al., 2016; Petrick et al., 2019). Furthermore, LIAE with BFR has not been found to alter autophagy, mitophagy, or genes implicated in substrate metabolism that represent metabolic stress during endurance exercise (Item et al., 2013; Smiles et al., 2017). Using a metabolomics approach, Valerio et al. (2018) reported greater levels of pyruvate and alanine following HLRE compared to LL-BFR RE, but similar acetoacetate and succinate. Christiansen (2018) assessed subunits of Na⁺/K⁺ ATPase and an ancillary protein phospholemman (FXYD) as well as components of Ca²⁺ signaling/handling and reported an increase in FXYD1 mRNA, no change in Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), but an increase in the ratio of phosphorylated phospholamban to total phospholamban—a
downstream target of CaMKII (Kuo & Ehrlich, 2015)—after running bouts with and without BFR.

**The Effects of BFR Exercise on Hypertrophic Mechanisms**

Provided exercise with BFR is predominantly conducted with the goal of muscle hypertrophy, thirteen (Aguayo et al., 2016; Barjaste et al., 2020; Conceicao et al., 2016; Ellefsen et al., 2015; Fry et al., 2010; Gundermann et al., 2012; Gundermann et al., 2014; Layne et al., 2017; Manini et al., 2011; Nyakayiru et al., 2019; Ozaki et al., 2014; Smiles et al., 2017; Wernbom et al., 2013) included papers evaluated changes in satellite cells, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) [e.g., extracellular signal-regulated kinase (ERK)1/2 and 38-kDa MAPK (p38 MAPK)] pathways, myofibrillar protein synthesis (MPS), myogenic regulatory factors [MRFs; e.g., myogenin, myoblast determination protein 1 (MyoD), myogenic factor 5 (Myf5)], proteolytic genes, and E3 ubiquitin ligases [e.g., forkhead box O3 (FOXO3A), muscle RING finger 1 (MuRF1), atrogin-1].

The PI3K/Akt/mTOR pathway has been found largely unaffected by the addition of BFR to LIAE (walking, cycling) (Conceicao et al., 2016; Ozaki et al., 2014), except for a decrease in eukaryotic translation elongation factor 2 (eEF2) phosphorylation (Ozaki et al., 2014) and an increase in Akt phosphorylation (Barjaste et al., 2020). In response to LL-BFR RE, increased phosphorylation of mTOR, Akt, 70-kDa ribosomal protein S6 kinase (p70S6K), ribosomal protein (rp)S6, and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) in the recovery period (1-3 hours post-exercise) have been found (Fry et al., 2010; Gundermann et al., 2012; Gundermann et al., 2014;
Nevertheless, this finding is not uncontested as Wernbom et al. (2013) found no changes in the PI3K/Akt/mTOR pathway after LL-BFR RE, except for an increase in p70S6K phosphorylation. Saliently, Fry et al. (2010) found no change in the tension-sensitive enzyme focal adhesion kinase (FAK) (Graham et al., 2015) after LL-BFR RE. Regarding MAPK signaling, increased phosphorylation of both ERK1/2 and p38 MAPK were reported after walking with BFR compared to without (Ozaki et al., 2014). This response was emulated after LL-BFR RE, with the additional increase in phosphorylation of MAP kinase-interacting serine/threonine-protein kinase 1 (MNK1) (Fry et al., 2010; Gundermann et al., 2012; Gundermann et al., 2014; Nyakayiru et al., 2019; Wernbom et al., 2013), a target of MAPK signaling (Fukunaga & Hunter, 1997; Waskiewicz et al., 1997). Nonetheless, Smiles et al. (2017) provided evidence of greater phosphorylation of p38γ MAPK after HLRE compared to LL-BFR RE. Lastly, MPS holistically increases after LL-BFR RE (Fry et al., 2010; Nyakayiru et al., 2019), an outcome posited to be independent of reactive hyperemia (Gundermann et al., 2012), yet at least partially dependent on mTOR (Gundermann et al., 2014).

Evaluation of the acute satellite cell response to LLRE divulged an increase in satellite cell quantity and number of MRF⁺ satellite cells per muscle fiber heedless of BFR, but a greater number of satellite cells with cytoplasmic extensions—that is, cells that appear elongated, thus indicating “activation”—only after LL-BFR RE (Wernbom et al., 2013). Additionally, Aguayo et al. (2016) found an increase in satellite cell quantity and frequency after WBV combined with BFR compared to non-BFR WBV, as well as a concomitant increase in quantity of frequency of myogenin⁺ myonuclei with BFR compared to without. These outcomes were noted in both type I and type II muscle fibers.
A novel finding by Layne et al. (2017) provides evidence of a decrease in mesenchymal epithelial transition factor (c-Met; also known as hepatocyte growth factor receptor) mRNA after LLRE with BFR compared to without, a HGF receptor insinuated to be requisite for satellite cell-mediated muscle regeneration (Webster & Fan, 2013). An additional unique finding by Ellefsen et al. (2015) was the comparable increase of syndecan-4 (SYND4) mRNA after HLRE and LL-BFR RE, a gene likewise associated with satellite cell function and regeneration (Cornelison et al., 2001; Cornelison et al., 2004).

The evidence of effects on MRFs and proteolytic enzymes is inconsistent. For instance, MuRF1 mRNA has been found to either increase or decrease following LL-BFR RE (Ellefsen et al., 2015; Gundermann et al., 2012; Manini et al., 2011). Accordingly, Manini et al. (2011) also reported a decrease in FOXO3A and atrogin-1 8-hours after exercise, but no changes in MRFs (MyoD, myogenin). Despite this decrease in FOXO3A mRNA, Fry et al. (2010) noted no change in phosphorylation of FOXO3A by LL-BFR RE. Findings by Layne et al. (2017) add further ambivalence as MyoD was found to increase following LLRE with BFR compared to that without, but no changes in Myf5 occurred.
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<th>Reference</th>
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<tr>
<td>Winchester et al. 2020</td>
<td>Resistance trained males (n=8) and females (n=4); age 21.7 ± 0.8 years</td>
<td>barbell back squat 5 × failure @75% 1RM w/ or w/o BFR 3-min rest</td>
<td>Unilateral (dominant leg) iBFR using 80% AOP</td>
<td>myoglobin, IL-6</td>
<td>↑ Myoglobin and IL-6 from baseline both, ↔ HLRE w/ or w/o BFR</td>
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<td>Behringer et al. 2018</td>
<td>Healthy physically active males (n=20; age 25.1 ± 3.1 years)</td>
<td>eccentric unilateral knee extensions 4 × failure @75% 1RM w/ or w/o BFR 30s rest</td>
<td>20 mmHg below AOP</td>
<td>CK, GH, IGF-1, lactate</td>
<td>↔ IGF-1, ↔ lactate, CK, GH HLRE w/ vs w/o BFR</td>
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<td>Neto et al. 2018</td>
<td>Resistance trained males (n=10; age 19 ± 0.8 years)</td>
<td>barbell bench press, pull down, triceps extension, biceps curl completed in order cBFR; 4 × 30-15-15-15 @20% 1RM 30s rest iBFR: 4 × 30-15-15-15 @20% 1RM 30s rest HLRE: 3 × 8 @80% unilater alternated knee extensions</td>
<td>iBFR and cBFR using 130% SBP</td>
<td>CK, LDH, UA, PC, TBARS</td>
<td>↑ CK in HLRE at 24-hr PE vs iBFR and cBFR; vs cBFR 48-hrs PE</td>
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<td>Cumming et al. 2017*</td>
<td>Untrained females (n=9; age 22 ± 1 years)</td>
<td>Tourniquet system set to 90 mmHg</td>
<td>Heat shock proteins, gene expression</td>
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<td>↔ HLRE &amp; LL-BFR RE overall mRNA expression</td>
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<td>↓ cytosolic αβ-crystallin, ↑ cytoskeletal, ↔ HLRE &amp; LL-BFR RE</td>
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<td>↑ cytoskeletal HSP27 both</td>
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| Cumming et al. 2014           | Physically active males (n=7) and females (n=2); age 26 ± 3 years                | Unilateral knee extension LL-BFR RE: 5 × failure @30% 1RM 45s rest LLRE: matched reps Tourniquet system set to 90 mmHg (females) or 100 mmHg (males) HSP27, HSP70, αβ-crystallin, desmin | ↓ Cytosolic HSP27 & αβ-crystallin BFR vs non-BFR 1-hr PE  
↑ Cytoskeletal HSP27 & αβ-crystallin BFR vs non-BFR 1-hr PE; αβ-crystallin 24-hr PE BFR vs non-BFR  
↑ Cytoskeletal HSP70 BFR vs non-BFR 24-hr PE  
↑ HSP70 in type 1 fibers BFR vs non-BFR; ↔ type 2 fibers  
↑ αβ-crystallin type 1 fibers, ↔ type 2 fibers  
↔ desmin |
| Wernbom et al. 2012           | Physically active males (n=8; age 26 ± 3 years) & females (n=4; age 24 ± 2 years) | Unilateral knee extension LL-BFR RE: 5 × failure @30% 1RM 45s rest non-BFR matched the sets & reps of BFR cBFR using 100 mmHg (males) or 90 mmHg (females) Intracellular tetranectin | ↑ tetranectin in muscle fibers BFR vs non-BFR at 24-hr PE  
↑ tetranectin in type 1 muscle fibers |
| Sieljacks et al. 2016*         | Non-resistance trained males (n=18; age 21 ± 0.6 years)                          | Knee extension LL-BFR RE: 5 failure @30% 1RM eccentric exercise: 15 × 10 maximal eccentric contractions via isokinetic dynamometer 45s rest cBFR using 100 mmHg | ↑ CK 24-, 48-, & 168-hr PE eccentric vs PrE  
↑ CK LL-BFR RE 48- and 96-hr PE vs PrE  
↑ myoglobin 1-hr & 96-hr PE in eccentric vs PrE; 48- & 96-hr PE in BFR vs PrE |
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<th>Maximal Concentric Power Output</th>
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<tr>
<td>Penailillo et al. 2020</td>
<td>Recreationally active males BFR n=10; age 24.9 ± 3.4 years non-BFR n=10; age 23.1 ± 3.0 years</td>
<td>Eccentric cycling @60 rpm for 30 min 60% maximal concentric power output</td>
<td>60% AOP</td>
<td>Lactate, CK</td>
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<td>↑ lactate BFR vs non-BFR</td>
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<td>↑ CK 48-hr PE vs PrE both, ↔ BFR vs non-BFR</td>
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<td>Dos Santos et al. 2020</td>
<td>Healthy, resistance trained males (n=20, age 18-36 years)</td>
<td>Bilateral leg press HLRE: 3 × failure @80% 1RM LL-BFR RE: 3 × 25 @40% 1RM 60s rest</td>
<td>cBFR using 80% AOP</td>
<td>Blood lactate, leukocytes, neutrophils, lymphocytes, monocytes, CK, cfDNA</td>
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<td>↔ blood lactate HLRE vs LL-BFR RE</td>
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<td>↑ cfDNA HLRE IAE vs PrE</td>
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<td>↑ CK in HLRE 24-hr PE vs PrE</td>
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<td>↑ Leukocytes, lymphocytes IAE vs PrE in both</td>
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<td>↔ Neutrophils, monocytes</td>
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<td>Dorneles et al. 2016</td>
<td>Untrained males (n=31, age 18-30 years)</td>
<td>Biceps curls and knee extensions HLRE: 4 × 8 @80% 1RM 2-min rest LL-BFR RE: 4 × 23 @30% 1RM 2-min rest</td>
<td>Biceps curl: 20 mmHg below SBP</td>
<td>CK, NK cells, CCR5</td>
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<td>Knee extensions: 20 mmHg above SBP</td>
<td>↑ CK both IAE vs PrE; 24-hr PE in HLRE vs PrE and LL-BFR</td>
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<td>↓ NK cells in HLRE 24-hr PE vs PrE and IAE</td>
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<td>↓ CCR5 24-hr PE vs Pre and IAE in HLRE; 24-hr PE vs IAE in LL-BFR RE</td>
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<td>Study</td>
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<tr>
<td>Shill et al. 2017</td>
<td>Active males (n=14; age 21.8 ± 0.4 years)</td>
<td>Hand grip dynamometer</td>
<td>bFGF, IL-6, IL-8, IL-10, TNF-α</td>
<td>↑ bFGF in BFR vs non-BFR at 5- and 30-min</td>
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<td>non-BFR: 1 contraction every 3s for 30 minutes w/ 65% MVIC</td>
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<td>↑ IL-6, IL-10, TNF-α BFR vs non-BFR at 30-min</td>
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<td>BFR: same as non-BFR w/ 20s deflation of pressure after every 5 min of exercise</td>
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<td>iBFR 95% SBP</td>
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<td>Nielsen et al. 2017*</td>
<td>20 recreationally active males [n=10 (BFR); age 23 ± 2 years; n=10 (HL); age 24 ± 3 years]</td>
<td>Unilateral knee extension</td>
<td>CK, GSH, TAC, MCP-1, IL-6, TNF-α</td>
<td>↑ CK in HLRE 180 min &amp; 24-hr PE vs PrE</td>
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<td>LL-BFR RE: 4 × failure @20% 1RM 30s rest</td>
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<td>↓ MCP-1 in LL-BFR RE 24-hr PE vs PrE</td>
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<td>HLRE: 4 × failure @70% 1RM 90s rest</td>
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<td>↔ IL-6 LL-BFR RE</td>
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<td>cBFR Set to 100 mmHg using a tourniquet system</td>
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<td>↑ IL-6 HLRE 24-hr PE vs PrE</td>
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<td>↔ TNF-α LL-BFR RE</td>
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<td>↓ TNF-α HLRE 180-min &amp; 24-hr PE vs PrE</td>
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<td>↔ TAC LL-BFR RE</td>
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<td>↑ TAC HLRE 15- &amp; 60-min PE vs PrE</td>
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<td>↔ GSH LL-BFR RE</td>
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<td>↑ GSH HLRE 5-min PE vs PrE</td>
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*Note: LL, left lower extremity; HL, right lower extremity; BFR, blood flow restriction; iBFR, intraarterial blood flow restriction; cBFR, cuff blood flow restriction; MVIC, maximum voluntary isometric contraction; SBP, systolic blood pressure; PE, post-exercise; PrE, pre-exercise; ±, plus or minus; 5- and 30-min, 5 and 30 minutes; 15-, 60-, and 5 min, 15, 60, and 5 minutes; IL-6, IL-8, IL-10, TNF-α, cytokines; ↑, increase; ↔, no change; 1RM, 1 repetition maximum.
<table>
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<tr>
<th>Study</th>
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<tr>
<td>Callanan et al. 2020</td>
<td>Healthy males (n=14; age 30.8 ± 3.7 years)</td>
<td>Knee extension, prone leg curl, leg press 4 × 30-15-15-15 @30% 1RM w/ or w/o BFR 30s rest</td>
<td>cBFR using 80% AOP using automated tourniquets</td>
<td>Leukocyte count, platelet count, neutrophils and lymphocytes, CD34⁺, lactate, glucose</td>
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<td>↑ CD34⁺ BFR IAE, ↑ platelet count both IAE, ↓ platelet count BFR 40-min PE, ↑ leukocytes IAE both, BFR vs non-BFR, ↑ lymphocytes BFR IAE, ↓ 60-min PE, ↓ neutrophils BFR IAE, ↑ 40- &amp; 60-min PE, ↑ lactate BFR vs non-BFR</td>
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<tr>
<td>Bugera et al. 2018</td>
<td>Resistance trained males (n=10; age 25.78 ± 3.56 years)</td>
<td>Bilateral knee extension LL-BFR RE: 4 × 30-15-15-15 @30% 1RM 30s rest</td>
<td>cBFR using 200 mmHg</td>
<td>IL-6, IL-15, decorin, No detectable levels of IL-6, ↔ IL-15, ↑ Decorin IAE both</td>
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<td>Cai et al. 2018</td>
<td>Untrained males (n=8; age 21.63 ± 1.19 years)</td>
<td>10 sets of WBV (1 minute each set) w/ or w/o BFR 1-min rest between sets, 2-min rest after 5th set</td>
<td>cBFR using 140 mmHg</td>
<td>GH, testosterone, ↑ blood lactate w/ BFR vs w/o, ↑ GH w/ BFR vs w/o, ↔ testosterone w/ BFR vs w/o</td>
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<td>Kraemer et al. 2016</td>
<td>Healthy males (n=8; age 21.8 ± 1.4 years)</td>
<td>Biceps curl &amp; calf raises LL-BFR RE: 3 × failure @30% 1RM 60s rest</td>
<td>cBFR using 20 mmHg below SBP (biceps curl) and 40 mmHg above SBP (calf raises)</td>
<td>Irisin, ↑ Irisin 15-min PE LL-BFR RE vs HLRE &amp; BFR w/o exercise</td>
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<td>Study</td>
<td>Intervention Details</td>
<td>Measures</td>
<td>Notes</td>
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<td>Behringer et al. 2017*</td>
<td>BFR (n=12 males; age 25.6 ± 2.3 years) Non-BFR (n=12 males; age 21.7 ± 2.1 years)</td>
<td>6 consecutive 100-m sprints @60-70% best sprint performance w/ 1-min rest between sprints w/ or w/o BFR</td>
<td>cBFR using elastic knee wraps pulled to 75% maximum GH, IGF-1, testosterone, cortisol, lactate, FABP ↔ lactate BFR vs non-BFR ↔ GH, IGF-1, testosterone, cortisol BFR vs non-BFR ↓ FABP IAE and 24-hr PE w/ BFR vs w/o</td>
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<td>Kim et al. 2014</td>
<td>Recreationally active females (n=13; age 21.5 ± 0.6 years)</td>
<td>Knee extension &amp; leg press LL-BFR RE: 3 × 30-15-15 @20% 1RM 60s rest HLRE: 3 × 10 @80% 1RM 60s rest</td>
<td>cBFR using 200 mmHg Lactate, GH, cortisol ↑ lactate HLRE vs LL-BFR RE ↔ GH, cortisol HLRE vs LL-BFR RE</td>
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<tr>
<td>Amani-Shalamzari et al. 2019*</td>
<td>Male futsal athletes (n=12; age 23 ± 2 years)</td>
<td>3-min futsal activity w/ 2-min rest, repeated 4 times w/ or w/o BFR</td>
<td>iBFR using 110% SBP IGF-1, myostatin ↔ IGF-1, myostatin IAE vs PrE w/ or w/o BFR</td>
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<tr>
<td>Amani-Shalamzari et al. 2020*</td>
<td>Male futsal athletes (n=12; age 23 ± 2 years)</td>
<td>3-min futsal activity w/ 2-min rest, repeated 4 times w/ or w/o BFR</td>
<td>iBFR using 110% SBP GH, testosterone, cortisol, lactate ↑ GH, testosterone, testosterone/ cortisol, lactate BFR vs non-BFR ↑ cortisol both IAE vs PrE</td>
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<td>Cook et al. 2014*</td>
<td>Resistance-trained males (n=20; age 21.5 ± 1.4 years)</td>
<td>Squat, bench press, pull-ups 5 × 5 @70% 1RM w/ or w/o BFR 90s rest</td>
<td>iBFR using 180 mmHg (lower limbs only) Testosterone, cortisol ↑ testosterone and cortisol w/ BFR vs w/o BFR</td>
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<tr>
<td>Study</td>
<td>Group Characteristics</td>
<td>Exercise Protocol</td>
<td>Training Protocol</td>
<td>C-BFR Pressure (mmHg)</td>
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<tr>
<td>Ozaki et al. 2017</td>
<td>Females (n=7; age 64 ± 2 years)</td>
<td>20-min walking w/ or w/o BFR @45% heart rate reserve</td>
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<td>Patterson et al. 2013</td>
<td>Physically active males (n=7; age 71.0 ± 6.5 years)</td>
<td>Unilateral knee extension 5 sets using individualized number of reps @20% 1RM w/ and w/o BFR 30s rest, matching reps between BFR &amp; non-BFR</td>
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<tr>
<td>Manini et al. 2012</td>
<td>Sedentary untrained males (n=10 young; age 28 ± 7.8 years, n=10 older; age 67.4 ± 4.6 years)</td>
<td>Knee extension LL-BFR RE: 4 × failure @20% 1RM 2-min rest HLRE: 4 × failure @80% 1RM 2-min rest</td>
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<td>150% SBP</td>
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<td>Inagaki et al. 2011</td>
<td>Recreationally active males (n=7; age 20.0 ± 0.7 years)</td>
<td>Isometric knee extension by EMS 3 × 40s-40s-60s @20% MVC w/ &amp; w/o BFR 30s rest</td>
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<td>150 mmHg</td>
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Table 3. (continued)

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<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Exercise Protocol</th>
<th>bFR</th>
<th>Parameters Assessed</th>
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<tr>
<td>Ozaki et al. 2014</td>
<td>Untrained males (n=6; age 22 ± 1 years)</td>
<td>20-min walking @55% VO$_2$max w/ or w/o BFR (unilateral BFR)</td>
<td>cBFR using 240 mmHg</td>
<td>Blood lactate, glucose, GH, cortisol, testosterone, IGF-1, Akt/mTOR &amp; MAPK cell signaling</td>
<td>↑ Lactate, GH, insulin ↔ Cortisol, IGF-1, testosterone ↑ ERK1/2-(P) BFR &amp; non-BFR ↑ p38 MAPK-(P) BFR ↔ Akt, mTOR, p70$^	ext{s6k}$-(P) in BFR or non-BFR ↓ eEF2-(P) BFR vs non-BFR 3-hr PE</td>
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<td>Conceicao et al. 2016</td>
<td>Untrained males (n=9; age 22.4 ± 3.0 years)</td>
<td>RE: 4 × 10 leg press @70% 1RM w/o BFR HIAE: 30-min cycling @70% VO$_2$peak w/o BFR LIAE: 15-min cycling @40% VO$_2$peak w/ BFR</td>
<td>cBFR using 80% SBP</td>
<td>Lactate, gene expression MuRF1, IL-6, COXIV, PGC1-α, HIF-1α, myostatin, IGF-1, cell signaling mTOR, p70$^	ext{s6k}$, AMPK, 4E-BP1, eEF2, p53</td>
<td>↑ PGC1-α HIAE w/o BFR vs LIAE w/ BFR, RE ↑ COXIV AE w/o BFR vs w/ BFR ↑ HIF-1α AE w/ BFR PE vs PrE ↑ MuRF1 AE w/o BFR vs w/ BFR, RE ↔ IL-6, IGF-1, myostatin, mTOR, p70$^	ext{s6k}$, AMPK, 4E-BP1, eEF2, p53 -(P)</td>
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<td>Fry et al. 2010</td>
<td>Physically active males (n=7; age 70 ± 2 years)</td>
<td>Bilateral knee extension 4 × 30-15-15-15 @ 20% 1RM 30s rest w/ and w/o BFR</td>
<td>cBFR using 200 mmHg</td>
<td>Cell signaling, gene expression, hormonal response, lactate, glucose, MPS</td>
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<td>↑ lactate, glucose, cortisol, GH</td>
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<td>BFR PE vs PrE, vs non-BFR</td>
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<td>↑ MPS BFR PE vs PrE, vs non-BFR</td>
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<td>↓ 4E-BP1-(P) IAE non-BFR</td>
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<td>↑ p70S6K-(P) IAE, 1-, 3-hr PE vs</td>
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<td>PrE BFR, vs non-BFR 3-hr PE</td>
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<td>↑ MNK1, rpS6-(P) non-BFR</td>
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<td>IAE vs PrE, MNK1 vs BFR</td>
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<td>↑ mTOR-(P) 1-hr BFR vs non-BFR, 3-hr PE both</td>
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<td>↑ rpS6 1- &amp; 3-hr PE BFR vs PrE, vs non-BFR 3-hr PE</td>
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<td>↔ HIF-1α, AMPK-(P), HSP70, IL-6, FAK-(P), FOXO3A-(P)</td>
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<td>↑ Akt-(P) BFR 3-hr PE vs PrE</td>
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<td>↑ ERK1/2-(P) 1-hr PE BFR vs non-BFR</td>
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<td>↑ MNK1-(P) 1-hr &amp; 3-hr PE BFR vs non-BFR</td>
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<td>Study</td>
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<td>Exercise Protocol</td>
<td>Training Protocol</td>
<td>Response</td>
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</table>
| Gundermann et al. 2012     | Recreationally active males (n=6; age 24 ± 2 years) | Knee extension 4 × 30-15-15-15 @20% 1RM 30s rest w/ BFR or w/ pharmacological hyperemia (vasodilation of femoral artery) | cBFR using 200 mmHg                                                                 | ↑ lactate BFR vs non-BFR  
↑ MPS BFR 3-hr PE vs PrE  
↑ Akt-(P) BFR 1-hr PE vs PrE  
↑ mTOR-(P) BFR vs non-BFR 3-hr PE  
↑ p70S6K-(P) BFR 3-hr PE vs PrE  
↑ rpS6-(P) BFR vs non-BFR 1-hr PE, 1 & 3-hr PE vs PrE  
↓ 4E-BP1-(P) non-BFR 3-hr PE vs PrE  
↑ MNK1-(P) BFR 3-hr PE vs PrE  
↔ eEF2, atrogin-1, MuRF1  
↑ MuRF1 mRNA BFR 3-hr PE vs PrE |
| Manini et al. 2011         | Recreationally active [males (n=8) and females (n=7); age 22.8 ± 3.7] years | Bilateral knee extension 4 × 30-15-15-15 @20% 30s rest 1RM w/ or w/o BFR | cBFR using 150% SBP                                                                 | ↔ IGF-1, MyoD, myogenin, myostatin  
↓ FOXO3A, atrogin-1, MuRF1 |
| Ellefsen et al. 2015*      | Untrained females (n=15; age 23 ± 3 years) | Unilateral knee extension LL-BFR RE: 5 × failure @30% 1RM 45s rest  
HLRE: 3 × 6-10 RM 90s rest | cBFR using 90 mmHg                                                                 | ↔ GH elevation BFR vs HL 1- & 30-min PE  
↑ cortisol BFR 1- & 30-min PE vs PrE  
↑ PGC1-α, MuRF1, SYND4 both  
↑ B-actin, REDD1 LL-BFR RE |
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<th>Activity Type</th>
<th>Exercise Details</th>
<th>Rest Details</th>
<th>CBF, BFR Parameters</th>
<th>Myofibrillar Protein Synthetic Rate &amp; Satellite Cell Activation</th>
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<tr>
<td>Gundermann et al. 2014</td>
<td>Recreationally active males (n=16; age 25.5 ± 0.8 years)</td>
<td>Bilateral knee extensions 4 × 30-15-15-15 @20% 1RM 30s rest w/ BFR w/ or w/o rapamycin ingestion</td>
<td>cBFR using 200 mmHg</td>
<td>MPS and breakdown, mTOR signaling</td>
<td>↑ MPS w/o rapamycin, ↔ w/ rapamycin</td>
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<tr>
<td>Nyakayiru et al. 2019</td>
<td>Physically active males (n=20, age 24 ± 1 years)</td>
<td>leg press and leg extension LLRE w/ and w/o BFR: 4 × 30-15-15-15 (leg press) and 3 × 10 (leg extension) @20% 1RM 30s rest Rest: 2 × 5 min BFR</td>
<td>cBFR using 200 mmHg</td>
<td>Myofibrillar protein synthetic rate, cell signaling, gene expression</td>
<td>↑ MPS LL-BFR RE vs non-BFR, ↔ rest vs rest+BFR, ↑ ACC-(P) BFR vs non-BFR, ↑ 4E-BP1-(P) BFR vs non-BFR at 2-hr PE</td>
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<td>Aguayo et al. 2016</td>
<td>Recreationally active males [n=25; age (WBV) 26.8 ± 1.2 years, (BFR) 27.7 ± 4.6 years, (WBV+BFR) 26.6 ± 4.0 years]</td>
<td>Static 135° knee flexion 3 × 4-min half-squat intervals 3-min rest w/ or w/o BFR, or BFR during rest</td>
<td>iBFR using 200 mmHg</td>
<td>Satellite cell activation</td>
<td>↑ satellite cell quantity and frequency in WBV+BFR PE vs PrE, ↑ myogenin+ myonuclei quantity WBV+BFR vs PrE, WBV, BFR, ↑ myogenin+ myonuclei frequency WBV+BFR vs PrE, WBV, ↑ myogenin+ myonuclei frequency type 1 and type 2 fibers BFR PE vs PrE</td>
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<td>Exercise Protocol</td>
<td>Circulation Restriction</td>
<td>Gene/Protein Expression</td>
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<td>Barjaste et al. 2020</td>
<td>Untrained males (n=5; age 33.41 ± 1.02 years)</td>
<td>5 × 2-min walking intervals @63-65% maximal heart rate 60s rest w/ and w/o BFR</td>
<td>cBFR using 200 mmHg</td>
<td>GH, IGF-1, cortisol, lactate, HIF-1α, PGC1-α, Akt</td>
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<td>↑ HIF-1α, PGC1-α BFR 3-hr PE vs PrE</td>
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<td>↑ Akt-(P) BFR 3-hr PE vs PrE and vs non-BFR</td>
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<td>↔ lactate</td>
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<td>↑ IGF-1 BFR IAE vs PrE and vs non-BFR</td>
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<td>↑ GH BFR vs non-BFR IAE</td>
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<td>↔ cortisol</td>
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<td>Layne et al. 2017</td>
<td>Untrained males (n=3) and females (n=3); age 22 ± 1 years</td>
<td>unilateral knee extension 10 × 12 @40% 1RM 60s rest w/ and w/o BFR</td>
<td>cBFR using 220 mmHg</td>
<td>Gene expression HGF, MyoD, Myf5, c-Met</td>
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<td>↑ MyoD BFR vs non-BFR 4-hr PE</td>
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<td>↓ c-Met mRNA BFR vs non-BFR 24-hr PE</td>
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<td>↓ HGF protein BFR vs non-BFR</td>
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<td>↔ Myf5</td>
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<td>Wernbom et al. 2013</td>
<td>Physically active males (n=6) and females (n=1); age 26 ± 1 years</td>
<td>unilateral knee extension LL-BFR RE: 5 × failure @30% 1RM 45s rest Non-BFR matched the sets &amp; reps of BFR</td>
<td>cBFR using 100 mmHg (males) or 90 mmHg (females)</td>
<td>Akt/mTOR signaling, MAPK signaling, satellite cells</td>
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<td>↑ p70S6K-(P) 1-hr PE in BFR vs PrE; 24-hr PE in both vs PrE</td>
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<td>↑ p38 MAPK-(P) BFR 1-hr PE vs PrE</td>
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<td>↑ satellite cells in both PE vs PrE</td>
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<td>↑ satellite cells w/ extensions in BFR</td>
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<td>Study</td>
<td>Condition</td>
<td>Exercise</td>
<td>BFR Protocol</td>
<td>Markers</td>
<td>Results</td>
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<td>Boeno et al. 2018</td>
<td>Physically active males</td>
<td>Bilateral elbow flexion and leg press</td>
<td>cBFR elbow flexion: 20 mmHg below SBP</td>
<td>NOx, SOD, CAT</td>
<td>↓ NOx in HLRE vs LL-BFR RE, ↓ SOD in LL-BFR RE IAE vs PrE, ↑ SOD in HLRE vs LLRE w/ and w/o BFR IAE, ↑ CAT in LLRE w/o BFR vs LL-BFR and HLRE at rest and IAE, ↓ CAT in LLRE w/o BFR IAE vs PrE</td>
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<td>(n=11; age 23.72 ± 3.49 years)</td>
<td>HLRE: 4 × failure @80% 1RM 60s rest</td>
<td>leg press: 20 mmHg above SBP</td>
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<td>LLRE w/ and w/o BFR: 4 × fail @30% 1RM 60s rest</td>
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<tr>
<td>Garten et al. 2015</td>
<td>Resistance trained males</td>
<td>Elbow flexion</td>
<td>cBFR 20 mmHg below SBP</td>
<td>PC, glutathione ratio, oxygen radical absorbance capacity, XO activity</td>
<td>↑ PC HLRE vs LLRE &amp; rest, ↑ glutathione ratio in HLRE vs LLRE &amp; rest, ↑ oxyg</td>
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<td>(n=12; age 25 ± 3 years)</td>
<td>LLRE w/ &amp; w/o BFR: 3 × failure @30% 1RM 60s rest</td>
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<td>spectral absorbance capacity in HLRE vs LLRE &amp; rest, ↑ oxygen radical absorbance capacity in HLRE vs LLRE &amp; rest, ↓ XO HLRE &amp; LLRE vs rest</td>
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<td>HLRE w/ &amp; w/o BFR: 3 × failure @70% 1RM 60s rest</td>
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<td>Rest w/ &amp; w/o BFR: rest for time matched to LLRE w/o BFR; w/ or w/o BFR</td>
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<td>Centner et al. 2019</td>
<td>Resistance trained males</td>
<td>120° knee flexion squats</td>
<td>cBFR using 60% AOP</td>
<td>Lactate, ROS</td>
<td>↑ lactate BFR vs non-BFR and control, ↔ ROS production</td>
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<td></td>
<td>(n=15; age 24.9 ± 3.5 years)</td>
<td>w/ and w/o BFR, control (no WBV); 3 × 120s, 60s rest</td>
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<td>Petrick et al. 2019</td>
<td>Healthy males</td>
<td>Single leg squats 3 × failure @30% 1RM 100s rest w/ &amp; w/o BFR</td>
<td>Tourniquet system using 60-70% AOP</td>
<td>Mitochondrial bioenergetics, ROS</td>
<td>↔ ETC protein content or antioxidant proteins, ↓ H₂O₂ emission LL-BFR RE</td>
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<td>(n=10; age 24 ± 1 years)</td>
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<td>Study</td>
<td>Group</td>
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<td>Training Factors</td>
<td>Outcome Measures</td>
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<td>Centner et al. 2018</td>
<td>Resistance trained males (n=15; age 24.8 ± 2.6 years)</td>
<td>Front squat</td>
<td>LLRE: 4 × 30-15-15-15 @30% 1RM 30s rest w/ &amp; w/o BFR</td>
<td>cBFR using 50% AOP</td>
<td>ROS, lactate ↑ systemic ROS production LL-BFR RE IAE vs PrE ↑ lactate HLRE vs LLRE w/ &amp; w/o BFR ↔ mitochondrial ROS production</td>
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<tr>
<td>Barili et al. 2018</td>
<td>Hypertensive females (n=16; age 67.2 ± 3.7 years)</td>
<td>HIAE: 50% estimated VO₂max</td>
<td>LIAE w/ &amp; w/o BFR: 30% estimated VO₂max</td>
<td>130% SBP</td>
<td>Oxidative stress ↔ NOx, PC ↑ TBARS HIAE, w/ BFR 30-min PE vs PrE ↑ GST HIAE 30-min PE vs PrE, LIAE 30-min PE vs IAE, w/ BFR 30-min PE vs IAE &amp; PrE ↓ NPSH w/ BFR 30-min PE vs PrE, w/ BFR &amp; HIAE vs w/o BFR ↑ SOD w/ BFR 30-min PE vs IAE &amp; PrE, vs w/o BFR</td>
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<td>Study</td>
<td>Participants Details</td>
<td>Exercise Protocol</td>
<td>BFR Protocol</td>
<td>Markers of Autophagy and MAPK Signaling</td>
<td>Gene Expression</td>
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<td>Smiles et al. 2017</td>
<td>Untrained males (n=9; age 22.4 ± 3.0 years)</td>
<td>HLRE: 4 × 10 leg press @70% 1RM (non-BFR) AE w/o BFR: 30 min cycling @70% VO2peak AE w/ BFR: 15 min cycling @40% VO2peak</td>
<td>cBFR using 80% SBP</td>
<td>↑ ULK1-(P) in AE w/o BFR vs rest &amp; AE w/ BFR ↓ Parkin in AE w/ BFR vs RE ↑ GSK3β RE vs AE w/ BFR ↑ GS-(P) AE w/ BFR vs w/o BFR &amp; RE ↑ p38γ MAPK-(P) RE vs AE w/ BFR ↑ GLUT4, HK2, AE w/o BFR vs w/ BFR &amp; RE ↑ PDK4 AE w/o BFR and RE vs AE w/ BFR</td>
<td>↑ PGC1-α, HK2 WBV+BFR vs w/o ↔ ERRα, NRF-1, Tfam, CS, LDHA, PFKm ↔ HIF-1α ↑ XD, MnSOD WBV+BFR PE vs PrE</td>
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<td>Item et al. 2013</td>
<td>Resistance trained males (n=8; age 23.1 ± 2.8 years)</td>
<td>Back squat 2 × failure @70% 1RM alone &amp; w/ BFR+WBV; 60s HLRE w/ or w/o BFR+WBV, 3-min BFR alone, 60s rest (no BFR, WBV, or HLRE)</td>
<td>iBFR using 200 mmHg</td>
<td>↑ PGC1-α, HK2 WBV+BFR vs w/o ↔ ERRα, NRF-1, Tfam, CS, LDHA, PFKm ↔ HIF-1α ↑ XD, MnSOD WBV+BFR PE vs PrE</td>
<td>↑ PGC1-α, HK2 WBV+BFR vs w/o ↔ ERRα, NRF-1, Tfam, CS, LDHA, PFKm ↔ HIF-1α ↑ XD, MnSOD WBV+BFR PE vs PrE</td>
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<td>Valerio et al. 2018</td>
<td>Resistance trained males (n=9; age 26.4 ± 4.4 years)</td>
<td>Leg press HLRE: 3 × 10 @80% 1RM 60s rest LL-BFR RE: 3 × 15 @20% 1RM 60s rest No exercise (CON)</td>
<td>cBFR using 80% SBP</td>
<td>↑ PGC1-α, HK2 WBV+BFR vs w/o ↔ ERRα, NRF-1, Tfam, CS, LDHA, PFKm ↔ HIF-1α ↑ XD, MnSOD WBV+BFR PE vs PrE</td>
<td>↑ PGC1-α, HK2 WBV+BFR vs w/o ↔ ERRα, NRF-1, Tfam, CS, LDHA, PFKm ↔ HIF-1α ↑ XD, MnSOD WBV+BFR PE vs PrE</td>
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<td>Christiansen et al. 2018</td>
<td>Recreationally active males (n=8; age 26 ± 5 years)</td>
<td>3 × 2-min running bouts (60s rest), repeated 3 times (5-min rest) @105% anaerobic threshold w/ BFR, w/o BFR, systemic hypoxia</td>
<td>cBFR using 3 mmHg·cm⁻¹ (relative to thigh circumference)</td>
<td>Gene expression, oxidative stress, metabolic stress</td>
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<td>↑ FXYD1 BFR 3-hr PE vs PrE</td>
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<td>↑ PGC1-α mRNA w/ BFR 3-hr PE vs PrE</td>
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<td>↑ catalase mRNA BFR</td>
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<td>↑ HSP70 mRNA BFR</td>
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<td>↑ lactate BFR and hypoxia</td>
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<td>↓ blood pH BFR vs non-BFR</td>
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<td>↔ PCr, PCr/Cr BFR</td>
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<td>↔ AMPK abundance or AMPK-(P) w/ &amp; w/o BFR</td>
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<td>↑ ACC-(P) BFR 1-hr PE vs PrE type 1 fibers</td>
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<td>↓ CaMKII-(P)/CaMKII type 2 fibers non-BFR IAE vs PrE</td>
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<td>↓ PLB abundance type 1 fibers non-BFR IAE vs PrE</td>
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<td>↑ PLB-(P)/PLB type 1 fibers BFR &amp; non-BFR, type 2 fibers</td>
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<td>BFR &amp; hypoxia IAE vs PrE</td>
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-(P) = phosphorylation ↑ = increased or greater than, ↓ = decrease or less than, ↔ = no difference or no change, * = only findings from acute data and first training session extrapolated, 1RM = one-repetition maximum, 4E-BPI = eukaryotic translation initiation factor 4E-binding protein 1, ACC = acetyl-CoA carboxylase, Akt = protein kinase B, AMPK = adenosine monophosphate-activated protein kinase, AOP = arterial occlusion pressure, bFGF = basic fibroblasts growth factor, BFR = blood flow restriction, c-Met == mesenchymal epithelial transition factor, CaMKII = Ca²⁺/calmodulin-dependent protein kinase II, CAT = catalase, cBFR = continuous BFR, CCR5 = C-C chemokine receptor type 5, CD34 = cluster of differentiation 34, cfDNA = circulating free DNA, CK = creatine kinase, COXIV = cytochrome c oxidase subunit IV, Cr = creatine, CS = citrate synthase, eEF2 = eukaryotic translation elongation
factor 2, EMS = electromyostimulation, ERK = extracellular signal-regulated kinase, ERRα = estrogen-related receptor alpha, ETC = electron transport chain, FABP = fatty acid binding protein, FAK = focal adhesion kinase, FOXO3A = forkhead box O3, FXYD1 = phospholemman, GH = growth hormone, GLUT4 = glucose transporter type 4, GS = glycogen synthase, GSH = glutathione, GSK3β = glycogen synthase kinase-3 beta, GST = glutathione S-transferase, HGF = hepatocyte growth factor, HIAE = high-intensity aerobic exercise, HIF-1α = hypoxia-inducible factor 1-alpha, HK2 = hexokinase 2, HLRE = high-load resistance exercise, HSP = heat shock protein, IAE = immediately after exercise, iBFR = intermittent BFR, IGF-1 = insulin-like growth factor-1, IL = interleukin, LDH = lactate dehydrogenase, LDHA = lactate dehydrogenase A, LIAE = low-intensity aerobic exercise, LL-BFR RE = low-load resistance exercise with BFR, LLRE = low-load resistance exercise, MAPK = mitogen-activated protein kinase, MCP-1 = monocyte chemoattract protein-1, MNK1 = MAP kinase-interacting serine/threonine protein kinase 1, MnSOD = manganese SOD, MPS = myofibrillar protein synthesis, mTOR = mechanistic target of rapamycin, MuRF1 = muscle RING finger 1, MVC = maximal voluntary contraction torque, MVIC = maximum voluntary isometric contraction, Myf5 = myogenic factor 5, MyoD = myoblast determination protein 1, NK = natural killer, NOx = nitrates and nitrites, NPSH = non-protein thiols, NRF-1 = nuclear respiratory factor 1, p38 MAPK = 38-kDa MAPK, p53 = tumor protein 53-kDa, p70S6K = 70-kDa ribosomal protein S6 kinase, p90RSK = 90-kDa ribosomal S6 kinase, PC = protein carbonyls, PCr = phosphocreatine, PDK4 = pyruvate dehydrogenase lipoamide kinase isozyme 4, PE = post-exercise, PFKm = phosphofructokinase, PGC1-α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha, PLB = phospholamban, PrE = pre-exercise, REDD1 = regulated in development and DNA damage response 1, ROS = reactive oxygen species, rpS6 = ribosomal protein S6, SBP = systolic blood pressure, SOD = superoxide dismutase, SYND4 = syndecan-4, TAC = total antioxidant capacity, TBARS = thiobarbituric acid reactive substances, Tfam = transcription factor A, mitochondrial, TNF-α = tumor necrosis factor alpha, UA = uric acid, ULK1= Unc-51-like autophagy activating kinase 1, VO2max = maximal oxygen consumption rate, VO2peak = peak oxygen consumption rate w/ = with, w/o = without, WBV = whole body vibration, XD = xanthine dehydrogenase, XO = xanthine oxidase
Discussion

**BFR Situationally Provokes Muscle Damage**

Studies indicating EIMD using the archetypal markers CK and myoglobin involve superimposition of BFR to HLRE (Behringer et al., 2018; Winchester et al., 2020), anomalously high training volumes and eccentric contractions (Sieljacks et al., 2016), eccentric cycling contractions (Penailillo et al., 2020), and untrained participants (Dorneles et al., 2016). By contrast, studies combining BFR with LLRE using trained participants and the recommended training volume of 75 total repetitions distributed through 3-4 sets holistically find a lack of change in the widely used blood markers of EIMD [e.g., CK, lactate dehydrogenase (LDH)] while non-BFR HLRE indeed results in expected EIMD (Dos Santos et al., 2020; Neto et al., 2018; Nielsen et al., 2017). Of note, Neto et al. (2018) and Dorneles et al. (2016) found an increase in CK immediately after LL-BFR RE, though this finding is unlikely related to EIMD as this diverges from the canonical time course of CK elevation (i.e., peaking at ~24-hr post-exercise and remaining elevated for 3-7 days, depending on the severity of damage) that would indicate EIMD (Coratella et al., 2016; Lee et al., 2017; Morán-Navarro et al., 2017; Pareja-Blanco et al., 2019; Sieljacks et al., 2016). These findings are coherent with the notion that the EIMD response is amenable to training status, unaccustomed physical exercise (e.g., training volume-load), and eccentric muscle contraction (Clarkson & Hubal, 2002; Ertel et al., 2020; Fridén & Lieber, 1992). Assessment of atypical markers of myocellular stress, including translocation of HSPs into cytoskeletal structures and the accumulation of intracellular tetranectin, indicate similar stress between HLRE and LL-BFR RE (Cumming et al., 2017; Cumming et al., 2014), as well greater indices of
perturbation after LL-BFR RE compared to non-BFR (Cumming et al., 2014; Wernbom et al., 2012) with the caveat that repetitions with BFR are conducted to failure. Intriguingly, LL-BFR RE ostensibly preferentially stresses type I muscle fibers (Cumming et al., 2014; Wernbom et al., 2012). This suggests LL-BFR RE results in muscle fiber stress in a different manner than HLRE, possibly related to prolonged tension and/or a metabolically stressful environment rather than through sheer mechanical tension. The collective findings indicate that exercise with BFR is capable of provoking EIMD, though the requirements for such as outcome seem stringent (figure 2).

Though only acute data was extrapolated for the purpose of this review, Cumming et al. (2017) found the acute translocation of HSPs was quelled after 12-weeks of training. This finding is consistent with the notion of susceptibility to EIMD based on training status, and further underscores the situational EIMD capability of LL-BFR RE. Therefore, it is conceivable that LL-BFR RE induces EIMD in a punctilious manner, necessitating equally fastidious assessment of participant training status and proper training volume-load when programming. This likely includes eschewing training to repetition-failure and/or using a high volume-load of eccentric contractions, particularly with untrained participants.
Figure 2. A compilation of the potential mechanisms driving hypertrophy after various forms of exercise with BFR. AE = aerobic exercise, Akt = protein kinase B, BFR = blood flow restriction, EIMD = exercise induced muscle damage, HIAE = high-intensity AE, HIF-1α = hypoxia-inducible factor 1-alpha, HLRE = high-load resistance exercise, IGF-1 = insulin-like growth factor 1, IL = interleukin, LL-BFR RE = low-load resistance exercise with BFR, MAPK = mitogen-activated protein kinase, MPB = myofibrillar protein breakdown, MPS = myofibrillar protein synthesis, mTOR = mechanistic target of rapamycin, REDD1 = regulated in development and DNA damage response 1, ROS = reactive oxygen species, RT = resistance training, STAT3 = signal transducer and activator of transcription 3, SYND4 = syndecan-4, w/ = with, WBV = whole body vibration.

BFR-Induced Inflammation is not Resultant of EIMD

Though the inflammatory response is considered staple in orchestrating muscle recovery after exercise (Tidball, 2017), EIMD and inflammation are not inextricable. The pro-inflammatory response to exercise is mediated in part by MCP-1 and TNF-α, cytokines that enhance resident neutrophil chemotaxis to the site of injury and engender an M1-biased macrophage phenotype to further augment phagocytosis (Peake et al., 2017; Shireman et al., 2007). As rejuvenation progresses from necrotic tissue ablation to myofiber healing, an anti-inflammatory response ensues to promote such tissue repair: a response partially modulated by IL-10 (Peake et al., 2017; Pedersen & Febbraio, 2008;
Tidball, 2017). Consistently, Shill et al. (2017) showed an increase in TNF-α 30-minutes during hand grip exercise with BFR, which is an early onset but at least superficially aligns with the purported time-course of TNF-α in response to injury (Peake et al., 2017; Tidball, 2017). However, Shill et al. (2017) also reported an increase in IL-10 at 30-minutes into exercise, which is much earlier than the expected response of this anti-inflammatory cytokine. This study is problematic in that the exercise session entailed 30-minutes of hand grip exercise with 1 contraction every 3 seconds. It can be speculated that the duration and metabolically stressful nature of the session as opposed to EIMD and inflammation is the culprit of increased IL-10 (Cabral-Santos et al., 2019). This notion can be justified by the commensurate increase in IL-6 observed in this study. Given that IL-6 is an energy status-acquiescent cytokine (Hennigar et al., 2017), and IL-10 is mediated by IL-6 (Peake et al., 2017; Steensberg et al., 2003), it is conceivable that the observed increase in IL-10 occurred sans EIMD or an inflammatory response related to muscle regeneration. This role of IL-6 is only briefly discussed in here as it is described further below (see “The Myokine and Hormonal Response to BFR is Attributable to Metabolic Stress”).

Continuing with other markers of inflammation, work by Nielsen et al. (2017) revealed no changes in TNF-α after LL-BFR RE and a decrease in MCP-1 24-hours post-exercise, potentially a result of an anti-inflammatory response (Peake et al., 2017; Tidball, 2017). Although only acute findings were extrapolated for the purpose of this review, Nielsen et al. (2017) also found a modest accumulation of macrophages in muscle three days after three weeks of LL-BFR resistance training (RT), apparently without appreciable indices of muscle damage/regeneration. This further supports the
inflammatory response pursuant to exercise with BFR occurs in lieu of substantial muscle fiber damage.

While the inflammatory response to EIMD is well described using changes in muscle tissue immune cells rather than peripheral blood, three of the included studies evaluated changes in blood leukocytes following LL-BFR RE (Callanan et al., 2020; Dorneles et al., 2016; Dos Santos et al., 2020). While the levels of leukocytes in the blood exhibit a high degree of variability contingent upon the exact parameters of exercise, the general consensus is that acute exercise stimulates all leukocyte types to ephemerally increase during or after stressful/damaging exercise by virtue of mobilization and migration to the injured muscle tissue (Gomes et al., 2020; Kawamura et al., 2018; Pedersen & Toft, 2000; Pizza et al., 1995; Wang et al., 2020). Congruently, leukocytosis, lymphocytosis, and an increase in CD34+ were found immediately after LL-BFR RE (Callanan et al., 2020; Dos Santos et al., 2020). However, Dos Santos et al. (2020) found no changes in neutrophils or monocytes after LL-BFR RE, possibly relating to the fact that the participants had experience in RE, as discussed shortly. Callanan et al. (2020) also showed a decrease in lymphocytes 60-minutes after LL-BFR RE, but an increase in neutrophils 40- and 60-minutes post-exercise. This effect may be related to lymphocytes egressing from circulation into muscle tissue (further considered shortly), and parallel neutrophil migration from sites of production to circulation in the post-exercise recovery period. Lastly, Dorneles et al. (2016) found a decrease in CCR5 24-hours after LL-BFR RE compared to levels immediately after exercise. This response may be reconciled by the untrained status of the participants. It can be postulated that the untrained participants harbor a more pronounced innate immune response and a reduction
in blood lymphocytes due to “immune surveillance”, a hypothesis suggesting exercise redeploy lymphocytes from circulation to peripheral tissues to identify and eliminate infected or damaged cells (Campbell & Turner, 2018). This disparity between studies based on training status is further fortified by the intimation that training develops the immune response, including “macrophage memory”—which enhances their response to injury (Tidball, 2017)—and dampening of the proinflammatory process (Peake et al., 2017), both of which may explain the lower magnitude immune response to LL-BFR RE in trained individuals.

In tandem, it can be speculated that the immune response to LL-BFR RE occurs due to factors other than EIMD, such as the degree of hypoxia or metabolic stress. Indeed, the appropriately monikered hypoxia-sensitive transcription factor HIF-1α is known to support immune cell function and provoke inflammation, a process that may be potentiated by perturbing cellular energetics (Krzywinska & Stockmann, 2018; Taylor & Colgan, 2017), as is thought to occur with BFR. To reiterate, local hypoxia and ensuing metabolic stress spurred by a reduction in blood flow and oxygen delivery by BFR may extend to resident immune cells within skeletal muscle tissue, thus augmenting their function. Further support of this hypothesis stems from the discovery of lactylation as a form of histone post-translational modification (Zhang et al., 2019). Specifically, these authors note an increase in histone lactylation in M1-biased macrophages, which are known to be more glycolytic in nature (Galván-Peña & O’Neill, 2014). Importantly, the increase in histone lactylation within M1-biased macrophages was concluded to be intrinsic (i.e., only affected by intracellular lactate production rather than lactate functioning in a paracrine fashion) and thus provides a meaningful role in resident
macrophages experiencing hypoxia. The consequential lactylation is important in the context of muscle hypertrophy as this study also found that genes primarily affected were involved in “wound healing”. Specifically, the gene for arginase 1 was among those affected, which is known to be expressed by M2-biased macrophages (Arnold et al., 2007; Deng et al., 2012; Mounier et al., 2013; Villalta et al., 2011; Villalta et al., 2009).

Therefore, histone lactylation within M1-biased macrophages may support the progression to the anti-inflammatory M2-biased phenotype, which in the case of muscle hypertrophy is necessary for adequate regeneration via myoblast differentiation and fusion as well as connective tissue production (Dort et al., 2019; Tidball, 2017) (figure 2). Therefore, the factors of hypoxia, metabolic stress, and attendant inflammation may not only partially account for BFR exercise-induced hypertrophy—considering the inflammatory response is known to ultimately promote satellite cell activation and fusion (Britto et al., 2020; Peake et al., 2017; Tidball, 2017)—but has practical application: the mild inflammatory response may allow for increased frequency of LL-BFR RE to promote rapid hypertrophy without risk of negative repercussions (e.g., excessive tissue injury and lack of repair). In fact, such protocols have been lucratively implemented (Bjørnsen et al., 2019; Nielsen et al., 2012; Nielsen et al., 2017). Given the extrapolated findings, the notion that LL-BFR RE likely results in minimal skeletal muscle damage but specifically warrants care in implementation more so in untrained individuals is further supported.

**The Addition of BFR Abates Oxidative Stress During Resistance Exercise**

Oxidative stress is an additional factor that potentially influences muscle hypertrophy through ROS production and cell signaling (Schoenfeld, 2013). Oxidative
stress constitutes a condition of an imbalance between the production and accumulation of ROS and the capacity of biological antioxidant defense systems to detoxify the reactive products (Pizzino et al., 2017). Under pathological conditions, excessive ROS production can lead to damage to various biomolecules, in turn damaging cells and tissue. However, there is a pivotal distinction between pathological ROS production and that ensued by exercise, the latter of which has various beneficial rather than harmful roles; this discussion is beyond the scope of this review but has been appropriately deliberated elsewhere (Simioni et al., 2018). The overall role of ROS in muscle hypertrophy is suppositional, through postulation that ROS production enhances MAPK signaling and IGF-1 function (Handyaningsih et al., 2011; Kefaloyianni et al., 2006). Nonetheless, training with the intent of generating high metabolic stress may enhance the production of ROS and subsequent hypertrophy-stimulatory pathways. However, it is currently suggested that high mechanical tension and muscle damage are the primary factors responsible for ROS production during and after RE (Pearson & Hussain, 2015; Schoenfeld, 2013), potentially due to the HLRE mediated recruitment of type II muscle fibers, which may be more inducive of ROS production (Quindry et al., 2011). Moreover, while exercise—more specifically, muscle contraction—alone is an accepted mode of augmenting intramuscular ROS production primarily via the mitochondrial electron transport chain, NADH-oxidase, and xanthine oxidase (Ji, 1999; Powers & Jackson, 2008; Powers et al., 2011), and bouts of hypoxia and/or ischemia/reperfusion are associated with an increase in ROS production (Clanton, 2007; Leurcharuskamree et al., 2018; Zuo & Clanton, 2005; Zuo et al., 2013), data coalesces to suggest no significant increase in oxidative stress occurs in response to LL-BFR RE (Boeno et al., 2018; Garten
et al., 2015; Neto et al., 2018; Nielsen et al., 2017; Petrick et al., 2019) or WBV with BFR (Centner et al., 2019). Of note, Centner et al. (2018) did show augmented oxidative stress in response to LL-BFR RE. This study was unique in directly assessing ROS production rather than the successive concentration of damaged biomolecules, and thus found an increase (0.074 ± 0.113 μmol/L/min) in systemic ROS production. It is possible that this low increase, albeit statistically significant, is not able to result in appreciable molecular damage (Mrakic-Sposta et al., 2012). The fact that only systematic ROS production increased while local values remained stagnant may also explain the lack of damage reported by prior research. Continuing, Barili et al. (2018) also found oxidative stress via an increase in lipid peroxidation, glutathione S-transferase, and superoxide dismutase activity, and a decrease in non-protein thiols during the recovery period (30-min) post-LIAE with BFR. The reason for these distinctive findings is likely related not only to a divergent mode of exercise compared to the other included papers (AE vs RE), but also to the use of hypertensive elderly female participants. Not only is AE alone a known stimulant of oxidative stress, especially in untrained individuals (Bouzid et al., 2015; Steinberg et al., 2007; Steinberg et al., 2006), but hypertension is associated with reduced antioxidant enzyme activity (Ahmad et al., 2013; Amirkhizhi et al., 2010), possibly resulting in their predisposition to increased activity during exercise. Christiansen et al. (2018) corroborates the findings of oxidative stress in response to AE with BFR as catalase, HSP27, and HSP70 mRNA increased after running bouts with BFR whereas this outcome was not seen after non-BFR. These combined findings point to the conclusion that oxidative stress is more so associated with the BFR combined with AE rather than BFR itself. Evidence provided by Item et al. (2013) also indicates that WBV
combined with HLRE (back squats) and BFR produces oxidative stress. However, these findings have little translation to common BFR implementation as this is a unique protocol. The augmented oxidative stress may be related to the HLRE, repetition time (4s eccentric, 1-2s eccentric to concentric transition, 4s concentric), the addition of WBV, sustained BFR after 60s of BFR and HLRE (3-min of BFR total, repeated twice), or a combination of all factors. These collective findings indicate, however, that perhaps the oxidative stress response to AE with BFR is a partial contributor to the noted muscle growth response to BFR walking protocols.

In contrast to AE with BFR, it may be that the addition of BFR to LLRE not only has no effect on ROS production but even decreases oxidative stress. Garten et al. (2015) found BFR blunts the oxidative stress response when added to LLRE compared to BFR at rest but increases oxidative stress when added to HLRE. This indeed supports the idea that high mechanical tension has a greater capacity to induce oxidative stress. The increase in oxidative stress during BFR at rest may be a result of stress to the vasculature rather than local skeletal muscle, as suggested by Garten et al. (2015). It is possible that the partial vascular occlusion resulted in appreciable venous blood pooling without exercise, resulting in stretching of the vasculature and subsequent ROS production (Birukov, 2009). The reason for an allayed oxidative stress response with the addition of BFR to LLRE may be explained by the skeletal muscle pump since this mechanism is suggested to contribute to both hyperemia and increased blood flow to the contracting tissue during exercise (Joyner & Casey, 2015). This process may have overcome the external compression of BFR and facilitated blood flow from the contracting muscle, limiting stretch on the vasculature, and lowering the rise and impact of ROS. In support of BFR as
means to assuage oxidative stress, Petrick et al. (2019) not only found no changes in oxidative stress in response to LL-BFR RE, but also found decreased mitochondrial H$_2$O$_2$ emission rates. The authors also conducted in vitro analysis to vet the effects of oxygen partial pressure on H$_2$O$_2$ emission rates, which confirmed lower oxygen partial pressure reduces ROS production. Taken together, the oxidative stress responses to LL-BFR RE seem to indicate that oxidative stress is not a driving factor behind muscle growth driven by this mode of exercise. Furthermore, this supplies supporting evidence that LL-BFR RE provokes marginal EIMD as ROS release and biomolecular damage are consequential to the neutrophil and M1-biased macrophage infiltration in response to injury (Peake et al., 2017), suggesting an excessive immune response indicative of EIMD does not occur. This lack of an excessive inflammatory response reinforces the use of LL-BFR RE at a higher training frequency than is capable with HLRE as the secondary damage from the immune response would plausibly be comparably diminutive.

**The Myokine and Hormonal Response to BFR is Attributable to Metabolic Stress**

Recent advancements in skeletal muscle physiology have identified the multifunctional tissue as a secretory organ, having the capability to release both cytokines (termed myokines) and extracellular vesicles (Rome et al., 2019), the former of which is esteemed as a potent regulator of skeletal muscle mass and function (Lee & Jun, 2019). The myokine response to exercise with BFR was therefore included in this review.

Traditionally, IL-6 was thought to primarily be involved in inflammation, exerting both pro- and anti-inflammatory effects, depending on the scenario (Pedersen et al., 2001). In an acute setting, this myokine is now known to be secreted from skeletal muscle and is an important regulatory molecule to maintain energy homeostasis (Lee &
and has been even posited to have a role in muscle hypertrophy (Serrano et al., 2008; Steyn et al., 2019). The discrepancies in the IL-6 response are most logically attributable to IL-6 being a low fidelity stimulant-specific marker—that is, IL-6 is used as a marker of EIMD but is highly responsive to metabolic stress—and the timing of sample collection. Studies indicting IL-6 increases after exercise with BFR sampled blood either during hand grip exercise with BFR (Shill et al., 2017) or in short recovery period (30-min) after LL-BFR RE (Patterson et al., 2013). Winchester et al. (2020) also indicated an increase in IL-6 60-min post-exercise, though this is more likely due to a pro-inflammatory response since BFR in this case was combined with HLRE, and both HLRE with and without BFR promoted an increase in IL-6. This increase in IL-6 shown by Shill et al. (2017) and Patterson et al. (2013) can therefore be presumed a product of the cytokine’s metabolism-regulatory functionality, providing evidence of exacerbated metabolic stress and further rebutting evidence for EIMD from LL-BFR RE (figure 2). On the other hand, studies showing no changes in IL-6 have taken samples 3-hrs after LIAE with BFR (Conceicao et al., 2016) or used older aged participants (Fry et al., 2010). In the former, this outcome may be related to the use of LIAE with BFR, which likely did not result in the inflammation-related elevation of IL-6 and the time of sampling was too long after exercise to represent metabolic stress. In the latter, the use of older adults may be the reconciling factor as aging is a known suppressant of the IL-6 response to exercise (Hamada et al., 2005; Toft et al., 2002; Windsor et al., 2018). Inert IL-6 values are corroborated by Nielsen et al. (2017) as no changes were found after LL-BFR RE (1- to 24-hr post-exercise) or HLRE (1- to 3-hr post-exercise), but an expected elevation 24-hr
after HLRE was shown. This finding suggests a lack of EIMD from LL-BFR RE considering the observed IL-6 response to HLRE is in agreement with the established upregulation 4- to 24-hr after HLRE (Louis et al., 2007). In aggregate, the studies support the position that LL-BFR RE is largely unable to induce EIMD, but instead results in substantial metabolic stress.

There is a paucity of findings on other myokines relative to the data on IL-6, with studies indicating LL-BFR RE increases decorin (Bugera et al., 2018) and irisin (Kraemer et al., 2016), both of which are implicated in muscle hypertrophy (Lee & Jun, 2019); however, IL-15—which related to muscle growth—was unaltered after LL-BFR RE (Bugera et al., 2018). Myostatin, a myokine involved in proteolysis, was found to be unaltered after AE with and without BFR (Amani-Shalamzari et al., 2019), HLRE, LIAE with BFR, and HIAE (Conceicao et al., 2016), and LLRE with and without BFR (Manini et al., 2011). These results may be related to the time of sampling or other methodological blunders as no response was seen in BFR or non-BFR exercise, which differs from the expected decline in myostatin mRNA in the 24-hour time period ensuing both AE and RE (Louis et al., 2007).

Although hormones are no longer considered to hold a premier role in muscle growth (at least within physiological ranges, that is) (Fink et al., 2018), the hormone response to exercise nonetheless may serve a subsidiary role in the hypertrophic process. To grossly encapsulate, exercise with BFR has been able to significantly elevate GH, either to a greater extent than lower-intensity non-BFR exercise or to a similar degree as higher-intensity exercise, though perhaps has no potentiating effect if added to high-intensity exercise (Behringer et al., 2017; Behringer et al., 2018). The GH response to
exercise with BFR may indicate augmented metabolic stress and represent a homoeostatic response to increase energy transfer to support cellular energetics as GH is a known stimulant of both lipid and glucose catabolism and is also prominently stimulated by highly metabolically stressful exercise (Gotshalk et al., 1997; Hoffman et al., 2003; Kim & Park, 2017; Kraemer et al., 1991; Kraemer et al., 1990; Kraemer et al., 2003; Moller et al., 2009). By contrast, GH also stimulates non-esterified fatty acid uptake into skeletal muscle (Bergan-Roller & Sheridan, 2018), where they—as now intramuscular fatty acids—may be metabolized to support muscle contraction or undergo re-esterification to triacylglycerol for future use. While overall consistent changes in GH were found, the lack of IGF-1 elevation is attributable to the timing of sampling as IGF-1 exhibits a delayed response that likely extends beyond the time breadths of acute analyses conducted in the included studies (Kraemer & Ratamess, 2005).

Both testosterone and cortisol are likewise implicated in energy homeostasis during exercise (Kraemer & Ratamess, 2005; Kraemer et al., 2020), aptly explaining the observed elevations of these hormones. There are, however, noteworthy differences between included studies. Akin to GH, numerous explanations for the enigmatic nature of testosterone and cortisol responses to exercise can be put forth since these hormones are receptive to differences in volume-load, intensity, diet, age, sex, training status, and muscle mass involved in exercise (Kraemer & Ratamess, 2005; Kraemer et al., 2020). Given the sizable heterogeneity between included studies (e.g., participant characteristics, exercise protocols), teasing out differences based on these variables is afflicted, especially considering the differences in sample collection methods/timing. However, it appears exercise intensity does in fact need to meet a minimum threshold to elicit
responses in testosterone or cortisol as this only occurred when BFR was combined with high-intensity exercise (Amani-Shalamzari et al., 2020; Behringer et al., 2017; Cook et al., 2014) and LL-BFR RE (Ellefsen et al., 2015; Fry et al., 2010; Kim et al., 2014). While Ozaki et al. (2017) found an increase in cortisol during walking with BFR, it is important to note the use of older adults as this protocol may have increased the level of physiologically perceived stress. If instead younger and/or trained participants were subjected to this low intensity protocol, such a response may not have ensued. This is further supported by elevated norepinephrine seen in this study as well. Finally, Inagaki et al. (2011) showed unchanged cortisol and norepinephrine after EMS with BFR, probably due to a sub-threshold exercise intensity.

In concert, the hormone responses to exercise with BFR, being acute measurements, are likely related to metabolic stress. While momentarily elevating anabolic hormone levels is no longer of upmost importance, perhaps designing BFR exercise protocols around aiming for these elevations is beneficial as this would indicate high metabolic stress, which in turn is thought to support muscle hypertrophy. Based on the combined hormonal and myokine findings, it seems, unsurprisingly, LL-BFR RE is the most potent mode of augmenting the hormonal response (which, in the context of metabolic stress-related muscle growth, would logically equate to greater hypertrophic outcomes). While HLRE has equal or superior effects in this regard, the basis of BFR is for use in those unable to tolerate higher external loads. Heretofore, BFR should be chiefly utilized alongside LLRE, with careful application of training to failure in untrained individuals (for expounded reasoning, see the preceding sections “BFR
Situationally Provokes Muscle Damage” and “BFR-Induced Inflammation is not Resultant of EIMD”).

**BFR Induces Notable Metabolic Stress**

Exercise with BFR reduces local arterial inflow and venous outflow from working musculature, and therefore logically limits oxygen delivery, generating intensified metabolic stress which in itself is thought to support muscle growth through a variety of elaborate mechanisms (Schoenfeld, 2013). Although lactate itself is surmised to have a dubious involvement in muscle hypertrophy and regeneration (Ohno et al., 2019; Oishi et al., 2015; Tsukamoto et al., 2018; Willkomm et al., 2014), given the widely agreed upon finding—well exceeding the contents of this review—of elevated blood lactate in response to exercise with BFR, exclusively molecular and otherwise novel findings will be discussed in more detail here. Briefly, however, it appears that if the goal is to increase blood lactate and potentially augment hypertrophy, rest periods between sets of LL-BFR RE should be as minimal as possible. Protocols that implemented either relatively longer rest periods (60-120s) between sets or relatively lower training volume-loads (non-failure training, lower than 4 × 30-15-15-15; discussed further shortly) found greater lactate concentration with HLRE versus LL-BFR RE (Kim et al., 2014; Manini et al., 2012; Valerio et al., 2018). The use of lower rest periods (30-45s) may potentiate lactate production, as 45s rest was found by at least one study to result in similar lactate values between HLRE and LL-BFR RE (Dos Santos et al., 2020). Of note, this protocol also required a total of 75 repetitions during LL-BFR RE, in this case through 3 × 25. This increase in lactate production is expected as the implementation of low rest periods between sets is rationalized to maximize metabolic stress (Kraemer & Ratamess, 2004;
Krzysztofik et al., 2019). However, low rest periods with the goal of increasing lactate may be at the expense of recovery of muscular force production capacity, thwarting performance during subsequent sets and/or exercises (Kraemer & Ratamess, 2004; Yasuda et al., 2013). This has potential to manifest as consistently lower mechanical tension over an exercise session and a training period, limiting the magnitude of the hypertrophic response seeing as mechanical tension is considered to be the driving mechanism of muscle hypertrophy (Bamman et al., 2018).

Continuing, one protocol of interest is that conducted by Valerio et al. (2018) which involved comparison of metabolites after HLRE and LL-BFR RE, better clarifying the differences in metabolic stress between these two modes of exercise. The finding of greater pyruvate, lactate, and alanine after HLRE compared to LL-BFR RE indicates an increased reliance on anaerobic ATP producing mechanisms in the former. The increase in pyruvate signifies supplemental reliance on glycolysis, lactate reflects elevated LDH mediated reduction of pyruvate in the cytosol to rejuvenate NAD⁺ for sustained glycolysis at the advent of mitochondria reaching metabolite “capacity” (Brooks, 2000; Brooks et al., 1999; Granchi et al., 2010), and alanine represents increased dependence on gluconeogenesis via the glucose-alanine cycle (Felig, 1973). Similar succinate and acetoacetate between exercise modes suggest comparable reliance on aerobic and fatty acid metabolism, respectively (i.e., the tricarboxylic acid cycle and oxidative phosphorylation; ketogenesis). Cohesively, this intimates that the decrease in oxygen delivery during LL-BFR RE is not sufficient in leading to a degree of metabolic stress reminiscent of HLRE. Only during high ATP demanding exercise—that is, exercise during which the rate of ATP demand cannot be satiated by aerobic ATP production
alone—is anaerobic ATP production ramped up to support an increase in ATP supply via substrate level phosphorylation (the phosphoglycerate kinase and pyruvate kinase catalyzed reactions) (Peek et al., 2017). Based on these findings, it seems LL-BFR RE is able to push mitochondria to their oxidative “capacity” and incur anaerobic ATP production, but not to the same extent as HLRE. However, this study is limited in the low percentage of sample analysis compared to the original sample size, and the use of a lower training volume-load LL-BFR RE exercise protocol (3 × 15) compared to the frequently used scheme of 4 × 30-15-15-15 (or training to failure), which perceptibly would promote greater metabolic stress. Therefore, the use of higher volume-loads or training to failure are plausible criteria for markedly enhanced metabolic stress during LL-BFR RE.

As previously conjectured, HIF-1α may have a clandestine role in the inflammatory and muscle hypertrophy responses to exercise with BFR. This hypothesis seems favorable when incorporating evidence that environmental hypoxia does indeed provoke inflammation and myogenesis via the IL-6/signal transducer and activator of transcription (STAT)3 pathway, evidently at the expense of elevated MPS (Britto et al., 2020; Etheridge et al., 2011; Gnimassou et al., 2018). However, it appears that HIF-1α is altered exclusively due to AE, a lackluster finding given this transcription factor is primarily known to be involved in endurance exercise-related adaptations (Lindholm & Rundqvist, 2016). Nonetheless, the effects of HIF-1α in BFR-mediated hypertrophy should not be completely ruled out as the use of untrained participants by Barjaste et al. (2020) and Conceicao et al. (2016) and the contrasting use of trained and active participants by Item et al. (2013) and Fry et al. (2010), respectively, may be a
confounding variable as the HIF-1α response is thought to diminish with exercise training (Lindholm & Rundqvist, 2016).

The energy-sensitive enzyme AMPK becomes catalytically active in a high AMP/ATP milieu and is therefore affected by bioenergetically taxing (e.g., high intensity, prolonged) exercise (Richter & Ruderman, 2009). Based on this, the effects of BFR exercise on AMPK have been scrutinized given that BFR apparently enhances local stress experienced by the exercising musculature. Strikingly, neither AE nor RE with or without BFR was able to upregulate AMPK or elevate AMPK phosphorylation, perhaps resultant of a lack of significant intensity and/or duration (Christiansen et al., 2018; Conceicao et al., 2016; Fry et al., 2010; Wernbom et al., 2013). However, ACC, an enzyme target of AMPK involved in the first step of fatty acid synthesis wherein acetyl-CoA is converted to malonyl-CoA (Lee et al., 2008), demonstrated greater phosphorylation after running bouts with BFR and LL-BFR RE (Christiansen et al., 2018; Nyakayiru et al., 2019). This post-translational modification would be beneficial in an exercising state as phosphorylation inhibits ACC activity (Kim, 1997), thus stymieing fatty acid biosynthesis and favoring catabolism for continued energy transfer (figure 3). In sight of dormant AMPK phosphorylation, it is possible that epinephrine is driving the increase in ACC phosphorylation in this case through the action of protein kinase A (PKA; also known as cAMP-dependent protein kinase). Though PKA has clear importance in signal transduction in both glucagon and epinephrine signaling to inhibit ACC (Haystead et al., 1990; Mabrouk et al., 1990) and is able to phosphorylate and inhibit ACC in hepatocytes (Holland et al., 1984; Sim & Hardie, 1988), PKA-stemmed
phosphorylation of ACC in skeletal muscle appears ineffective (Winder et al., 1997). Therefore, the origin of increase in ACC phosphorylation remains to be unveiled.

Further complicating findings, an increase in PGC1-α mRNA has been found after various forms of exercise with BFR (Barjaste et al., 2020; Christiansen et al., 2018; Ellefsen et al., 2015; Item et al., 2013), except low-intensity cycling with BFR (Conceicao et al., 2016). Given PGC1-α is a regulator of mitochondrial biogenesis and promotes muscle fiber shift to a more oxidative phenotype (Lin et al., 2002; Lira et al., 2010) and PGC1-α mRNA is responsive to endurance exercise (Baar et al., 2002; Goto et al., 2000; Irrcher et al., 2003; Pilegaard et al., 2003; Sadana et al., 2007; Terada et al., 2002), it seems logical that mRNA abundance would increase after metabolically taxing BFR exercise. The interaction between AMPK and PGC1-α is not well elucidated, but may involve p38 MAPK (Puigserver et al., 2001; Wu et al., 2015) and action of transcription factor EB, which binds to and activates the promoter of the PGC1-α gene (Settembre et al., 2013) (figure 3). In the case of p38 MAPK, this may be indirectly supported by studies included in this review showing an increase in p38 MAPK phosphorylation in response to exercise with BFR (Ozaki et al., 2014; Wernbom et al., 2013), delineated further in “Exercise with BFR Supports Muscle Hypertrophy in a Distinctive Manner,” below. However, Smiles et al. (2017) indicated an increase in p38γ MAPK [suggested to have a greater role in exercise-induced adaptations than other p38 MAPK isoforms (Lira et al., 2010)] and Unc-51-like autophagy activating kinase 1 (ULK1) [a downstream target of AMPK signaling involved in autophagy (Laker et al., 2017)] phosphorylation in response to HLRE and HIAE, but not LIAE with BFR. This discrepancy may be related to a plethora of variable interactions aside from the more
obvious effects of divergent muscle biopsy sampling times. For example, Wernbom et al. (2013) required LL-BFR RE performed to repetition failure, potentially providing sufficient stimulus for augmented metabolic stress above that of low-intensity cycling with BFR as conducted by Smiles et al. (2017). While Ozaki et al. (2014) implemented BFR walking, BFR was applied at 240 mmHg while Smiles et al. (2017) used 80% AOP, coinciding to ~90 mmHg. The excessively high pressure may be the source of the drastically augmented metabolic stress during BFR walking conducted by Ozaki et al. (2014). Collectively, downstream effects of AMPK provide evidence in favor of augmented metabolic stress during exercise with BFR. Though AMPK phosphorylation and abundance were not altered, this may be a fault of timing of muscle biopsy sampling as the subsequent signaling processes, at least with the evidence within the realm of this review, seem intact. Based on exercise variables, a significantly high degree of metabolic stress to influence AMPK signaling may involve high-intensity exercise with BFR, LL-BFR RE to failure, or extraordinarily high occlusive pressures. Provided the potential drawbacks of using high pressures (e.g., elevated discomfort and risk of reduced compliance to a training program) and training to failure (especially in certain at-risk populations) and the counterintuitive nature of combining BFR with high-intensity exercise, the discretion of practitioners is ultimately the final factor ascertaining the risk-reward benefit of perusing this response solely from a molecular basis. Sensibly, LL-BFR RE to failure or BFR combined with high-intensity exercise in the interest of endurance exercise-related adaptations may be supported exclusively in trained individuals and without abnormally high training frequencies, as per information provided in prior sections.
Some distinct variables assessed by individual studies are briefly discussed here for their relevance in better understanding the role of BFR in endurance related adaptation. First, Smiles et al. (2017) posited that the changes in mRNA of glucose transporter type 4, pyruvate dehydrogenase lipoamide kinase isozyme 4, and hexokinase 2 reflect an acute energy restoration process, providing evidence that HIAE is more stressful than LIAE with BFR in their protocol. The authors continue to conclude that LIAE with BFR in unable to alter energy status enough to galvanize autophagy and therefore is not as effective in inducing endurance related adaptation as standard HIAE since neither ULK-1 nor Parkin were upregulated in response to the BFR protocol, both of which are involved in autophagy (Ghosh et al., 2020). This also provides evidence against a substantial degree of hypoxia or ROS production as both of these factors are damaging to mitochondria, which in turn promote autophagy through an intricate mechanism previously described (Ghosh et al., 2020; Onishi et al., 2021). Conversely, Christiansen et al. (2018) found an increase in FXYD1 mRNA after HIAE with BFR, a response the authors suggest being resultant of increased ROS production, as discussed prior. This observation is paramount for endurance related adaptation as an increase in ROS production after prolonged or high-intensity AE is implicated in endurance exercise adaptations (e.g., increase in skeletal muscle antioxidants, mitochondrial biogenesis) through nuclear factor kappa-light-chain-enhancer of activated B cells and PGC-1α signaling (Powers et al., 2020), the latter of which was shown responsive to HIAE with BFR. These findings at a superficial level indicate differences in the ability of AE with BFR to promote endurance exercise adaptations. Though different outcome variables were assessed in these studies, divergent author conclusions are attributable to the use of
LIAE with BFR by Smiles et al. (2017) versus HIAE with BFR by Christiansen et al. (2018). Thus, it may be superimposition of BFR to HIAE is requisite for endurance related adaptations in a physically healthy population. Overall, responses to exercise are related to the intensity, duration, and mode of exercise, providing difficulty in reconciling differences due to equivocal comparison of, for instance, low-intensity cycling, low-intensity, walking, and running bouts.

**Figure 3.** Summary of the acute endurance exercise-related responses to various forms of exercise with BFR. AC = adenylyl cyclase, ACC = acetyl-CoA carboxylase, AE = aerobic exercise, AMPK = adenosine monophosphate-activated protein kinase, ATP = adenosine triphosphate, BFR = blood flow restriction, cAMP = cyclic adenosine monophosphate, CoA = coenzyme A, HIAE = high-tensity aerobic exercise, LL-BFR RE = low-load blood flow restriction resistance exercise, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells, p38 MAPK = 38-kDa mitogen-activated protein kinase, PGC1-α = PPAR gamma coactivator 1-alpha, PKA = protein kinase A, PPARGC1A = peroxisome proliferator-activated receptor gamma coactivator 1-alpha, ROS = reactive oxygen species, TFEB = transcription factor EB.
Exercise with BFR Supports Muscle Hypertrophy in a Distinctive Manner

Out of respect for the oscillatory nature of gene expression, it is prudent to consider the ensemble of findings rather than papers in isolation. Canonical muscle growth cell signaling cascades include the PI3K/Akt/mTOR pathway and MAPK signaling. In the former, upon phosphorylation by Akt, mTOR phosphorylates and inhibits 4E-BP1, facilitating translation initiation by permitting the 5’ 7-methylguanosine “cap” recognition action of eukaryotic translation initiation factor (eIF)4E and assembly of the eIF4F complex on the target mRNA (Grifo et al., 1983; Grüner et al., 2018; Haghhighat & Sonenberg, 1997; Igreja et al., 2014; Matsuo et al., 1997; Peter et al., 2015). Phosphorylation of p70\textsuperscript{S6K} supports translation and a greater translational capacity through successive phosphorylation of rpS6, a component of the 40S ribosomal subunit (Ferrari & Thomas, 1994; Terada et al., 1994). Indeed, S6 kinases (e.g., p70\textsuperscript{S6K}) appear to have an influence on ribosome biogenesis (Chauvin et al., 2014) and, in the context of muscle fiber hypertrophy, ribosome biogenesis is a major contributor to the translational capacity of the cell and thus maintains an essential role in the control of growth (Chaillou et al., 2014).

Eukaryotes maintain multiple MAPK pathways, four of which are principally involved in skeletal muscle signaling (Cargnello & Roux, 2011; Kramer & Goodyear, 2007), and two of the four, known as ERK1/2 and p38 MAPK, have been investigated in the included eligible studies. The involvement of these signaling cascades in skeletal muscle cells are presently equivocal, but likely involve the proliferation of satellite cells and, within the muscle fibers themselves, downstream stimulation of protein synthesis by phosphorylating rpS6 and the amplified activity of a variety of substrates including MRFs.
(i.e., MyoD and myocyte enhancer factor-2) (Jones et al., 2001; Keren et al., 2006; Murgia et al., 2000). However, as previously alluded to, the MAPKs are also largely involved in substrate metabolism and a shift to an oxidative muscle fiber phenotype, providing impetus for evaluation during highly metabolically taxing BFR exercise (Kramer & Goodyear, 2007).

Although BFR walking training programs have been found to elicit muscle hypertrophy (Abe et al., 2006; Abe et al., 2010; Ozaki et al., 2011; Sakamaki et al., 2011), acute responses exhibited minor changes in both PI3K/Akt/mTOR and MAPK signaling pathways (e.g., decreased eEF2 phosphorylation and increased Akt, ERK1/2, and p38 MAPK phosphorylation) (Barjaste et al., 2020; Ozaki et al., 2014). By contrast, LL-BFR RE seems to considerably embolden myofibrillar protein synthetic cell signaling, evident by more pronounced effects on PI3K/Akt/mTOR and MAPK signaling cascades compared to both walking with BFR and non-BFR LLRE. This is a rather lackluster finding as greater hypertrophy from LL-BFR RE compared to AE with BFR is expected, since this would rationally be consistent with greater muscle growth from RE versus AE in general (Grgic et al., 2019). Furthermore, MAPK pathways appear to be stimulated by local rather than systemic stress, including intensity and cellular energy status (Aronson et al., 1997; Kramer & Goodyear, 2007), explaining greater activation in response to LL-BFR RE compared non-BFR LLRE and walking with BFR. Oxidative stress may be the culprit of the MAPK response to AE with BFR as MAPK signaling is thought responsive to ROS (Son et al., 2013; Zhang et al., 2016), suggesting different mechanisms of action between AE and RE but nonetheless supporting the use of BFR in both.
While it is no surprise MPS rates increase after LL-BFR RE, consistent with “activation” of the aforementioned pathways, Gundermann et al. (2012) provided evidence against the hypothesis that hyperemia is a significant contributor to hypertrophy during BFR exercise. An additional key finding is the lack of change in FAK phosphorylation after LL-BFR RE (Fry et al., 2010), a fundamental component of hypertrophy by virtue of mechanotransduction. Although only interrogated by a single study, this perhaps provides evidence that LL-BFR RE promotes hypertrophy without stimulating tension-sensitive mechanisms as HLRE putatively does (Bamman et al., 2018).

As previously mentioned, the role of hypoxia in BFR-induced hypertrophy is elusive, and it may even be that this local environment acutely inhibits MPS. This notion is supported by an increase in mRNA of the hypoxia-responsive protein regulated in development and DNA damage response 1 (REDD1) 1-hr after LL-BFR RE to failure (Ellefsen et al., 2015), as REDD1 is known to inhibit mTOR signaling (DeYoung et al., 2008). Yet, with respect to the holistic increase in MPS after LL-BFR RE, this effect is likely minimal. Importantly, REDD1 is also thought to induce inflammation (Pastor et al., 2017), a finding that merits discussion. Based on the available evidence previously deliberated, it can be speculated that rapid hypertrophy resultant of a BFR training program is driven by mild inflammation and subsequent satellite cell activation, supported by the upregulation of REDD1 after stressful (training to failure) LL-BFR RE (figure 2). This increase in satellite cell activation is acutely supported by the observed increase in SYND4 expression after LL-BFR RE to failure (Ellefsen et al., 2015) as this protein demonstrably has a role in satellite-cell mediated muscle regeneration (Cornelison
et al., 2001; Cornelison et al., 2004), evidently in response to EIMD (Casar et al., 2004) (figure 2). This also provides supporting evidence that LL-BFR RE to failure likely results in some degree of muscle damage, the magnitude of which not reaching a detectable threshold by the regularly used markers and/or does not result in substantial ultrastructural damage. Regardless, examination of the increase in activation and content of satellite cells have indeed favored BFR after both LLRE and WBV (Aguayo et al., 2016; Wernbom et al., 2013).

Referring back to hypoxia, this environment may explain the observed decrease in HGF protein content and c-Met mRNA after high volume-load LL-BFR RE in untrained participants (Layne et al., 2017). Hepatocyte growth factor is the ligand for the receptor tyrosine kinase c-Met, and HGF/c-Met signaling is involved in both embryonic myogenesis and adult skeletal muscle regeneration through influence on myoblast migration and fusion (Anastasi et al., 1997; Gal-Levi et al., 1998; Webster & Fan, 2013). Therefore, the downregulation of both HGF and c-Met seemingly signifies a reduced hypertrophic/regenerative capacity after LL-BFR RE in this study. The observed decrease in HGF may be attributed to local hypoxia as this stimulus has been shown to blunt the HGF response in vitro (Flann et al., 2014). Moreover, the decrease in c-Met mRNA is to be expected as a positive feedback loop appears to exist in the HGF/c-Met pathway (i.e., without high downstream substrate concentrations, c-Met is not substantially upregulated), likely involving STAT3 (Syed et al., 2011); though this remains to be explored specifically in skeletal muscle. Accepting the inflammatory response to exercise with BFR as an important factor in muscle growth, the blunted HGF response has important application as HGF/c-Met signaling is thought to have a role in the transition of
macrophages to an M2-biased phenotype (Choi et al., 2019). Nevertheless, Layne et al. (2017) still found an increase in MyoD mRNA 4-hr after LLRE with BFR compared to without, yet no differences in protein content of myf5 or MyoD were observed between BFR and non-BFR. In all included papers, this was the only case of an increase in MyoD after exercise with BFR, likely relating to the timing of sampling. Holistically, it appears exercise with BFR does not significantly alter myogenic genes acutely (1-8 hr after exercise). This reveals a dissimilarity between LL-BFR RE and HLRE as the former is unable to significantly alter MRFs in the normal timeframe of the myogenic gene response to HLRE (between 2-12 hours post-exercise) (Yang et al., 2005). Additionally, the sole response of MyoD is consistent with a prior documented response to AE (Yang et al., 2005), suggesting LL-BFR RE is more redolent of AE in this specific regard. Nonetheless, the relevance of these findings is unclear as the similarities between HLRE and LL-BFR RE from a physiological stress standpoint are not cogent. Despite superficial comparability to HLRE, LL-BFR RE has some mechanistic characteristics of both AE and HLRE, though it reigns superior to AE with BFR in promoting hypertrophy.

Differing from the myogenic genes, proteolytic genes appear to exhibit a more rapid response to exercise with BFR. Specifically, the responses of the E3 ubiquitin ligases MuRF1 and atrogin-1 have been evaluated due to their role in myofibrillar protein breakdown via the ubiquitin-proteasome pathway; a process involving polyubiquitination of proteins by these ligases, thus targeting myofibrillar proteins for degradation by the 26S proteasome (Reid, 2005). Additionally, the mRNA content and phosphorylation of FOXO3A were also evaluated as this is a transcription factor of both MuRF1 and atrogin-1 (Sandri et al., 2004; Stitt et al., 2004), and phosphorylation at serine 253 by Akt is
known to exclude FOXO3A from the nucleus and therefore hinder its activity (Brunet et al., 1999; Dobson et al., 2011; Tzivion et al., 2011). While solitary myofibrillar protein breakdown seems reasonably counterproductive when envisaging muscle hypertrophy, coordinated MPS and degradation are requisite for muscle remodeling and adaptation, which is an established response to HLRE (Kumar et al., 2009). Evaluation of these outcomes indicates exercise with BFR promotes myofibrillar protein breakdown (via upregulation of MuRF1) within 1-4 hr post-exercise (Ellefsen et al., 2015; Gundermann et al., 2012), with a similarly expected decrease in MuRF1, atrogin-1, and FOXO3A mRNA 8-hr after exercise (Manini et al., 2011). Importantly, this response mimics that of traditional HLRE, but differs from the previously documented response to HIAE (Louis et al., 2007). This further indicates that LL-BFR RE is indeed more resemblant of HLRE than HIAE from a net protein balance and exercise adaptation standpoint, which is to be expected as the mode of exercise is the same. This information is nonetheless useful in that though LL-BFR RE utilizes a repetition range associated with “muscular endurance training” and is highly metabolically taxing, the remodeling processes is presumably at least somewhat analogous to that of HLRE, possibly supporting a hypertrophic outcome (figure 2). Indeed, the similar time course of accelerated proteolysis following LL-BFR RE and HLRE bolsters the use of BFR as a valid substitute.

Considering contradictory data, Fry et al. (2010) found no changes in FOXO3A phosphorylation after LL-BFR RE, explicable by the timing of sampling (3-hr after exercise), leaving the possibility that detectable modifications were not prominent at this time point. Provided the previous observation that FOXO3A mRNA decreases 8-hr after exercise during both HLRE and apparently LL-BFR RE, it is conceivable that
phosphorylation and consequent inhibition of nuclear localization does not occur this early in the recovery period after exercise. The observation of an increase in Akt phosphorylation abjures this hypothesis as FOXO3A is a target of Akt, yet it is possible that alternative FOXO3A phosphorylation and inhibition occurs via MAPK signaling, which would support a positive protein balance, myofibrillar protein accrual, and eventual muscle hypertrophy. Accordingly, Fry et al. (2010) found an increase in ERK1/2 phosphorylation which is valuable as ERK is in fact capable of phosphorylating and inhibiting FOXO3A (Yang et al., 2008). However, phosphorylation by this pathway alternatively occurs at serine 294, serine 344, and serine 425 while Fry et al. (2010) only investigated serine 253 phosphorylation and did not quantify the rate of myofibrillar protein breakdown; thus, the contribution of this effect is conjectural and equivocal. Keeping in mind the conspicuous lack of change in AMPK after exercise with BFR (despite the notable downstream effects), this can be considered beneficial from a hypertrophic standpoint. Prior research has indicated AMPK can phosphorylate FOXO3A at threonine 179, serine 399, serine 413, serine 555, serine 588, and serine 626, which enhances FOXO3A activity without affecting subcellular localization, potentially through the formation of a transcriptional complex and directing FOXO3A to promotor regions (Greer et al., 2007). Since AMPK is responsive to prolonged endurance exercise and endurance exercise is associated with prolonged FOXO3A mediated-proteolysis, this is an additional source of evidence favoring LL-BFR RE as a substitute for HLRE despite overt exercise protocol similarities to exercise with the goal of increasing muscular endurance (i.e., high-repetition exercise).
Solely based on acute findings, this evidence venerates various forms of exercise with BFR as potent stimuli for satellite cell activation and similar cell signaling responses, MPS, and proteolysis as HLRE (except FAK signaling, as anticipated), all of which presumably support and are the driving mechanisms behind muscle hypertrophy. Though findings from training studies were not included in the formal literature search, the association between acute and chronic findings should be considered for additional relevance and application. For instance, in the case of satellite cells, Ellefsen et al. (2015) found that myonuclear number per muscle fiber was unaltered after 12-weeks of LL-BFR RT. Nevertheless, myonuclear number was also unchanged after HLRE, whereas other chronic studies (Bjørnsen et al., 2019; Nielsen et al., 2012) have found a substantial increase in myonuclei. The difference between these studies primarily lies in the frequency of training sessions, insinuating that higher frequencies of LL-BFR RE are necessary to promote this rapid hypertrophy. However, this may be contraindicated in certain populations as the high frequency of exercise sessions may lead to negative side effects, both at the tissue (excessive stress/damage) and perceptual (soreness) levels, that would deter individuals from complying to an exercise program. This insinuation is strengthened by the observation of an initial decrease in muscle fiber size after high frequency LL-BFR RE to failure prior to delayed hypertrophy following a LL-BFR RT intervention in non-resistance trained participants (Bjørnsen et al., 2019). Ostensibly, lower-intensity exercise with BFR (AE, LL-BFR RE not to failure) may suffice in increasing acute hypertrophic outcomes in untrained individuals, whereas perhaps higher intensity exercise can be conducted safely in trained individuals to bolster the hypertrophic effect. This risk-reward ratio is again at the discretion of individual
practitioners; based on both acute and chronic evidence, however, it appears higher frequencies of LL-BFR RE (perhaps without performing repetitions for failure) would be a key component of a lucrative muscle hypertrophy-oriented program.

Conclusions and Application to Exercise Programming

Exercise with BFR has shown increasing popularity in both rehabilitative and recreational settings. However, proper implementation within a training program has yet to be established. Evaluation of acute responses to exercise with BFR provides valuable insight not only into mechanisms of observed muscle hypertrophic outcomes, but also uncovers key variations in training that can be manipulated to optimize a training program. The findings presented here indicate a few key points: 1) LL-BFR RE is in fact able to induce meaningful muscle fiber stress provided training is conducted to failure, and untrained participants are at increased risk of this low-grade EIMD; 2) an inflammatory response to exercise with BFR is evident, though this is unlikely related to EIMD and is similarly more pronounced in untrained participants; 3) RE with BFR rescinds oxidative stress, while AE with BFR facilitates this response provided sufficient intensity; 4) the hormonal response to BFR exercise is likely linked to metabolic stress, and metabolic stress is indeed prominent during BFR exercise and is exacerbated during high-intensity exercise with BFR, LL-BFR RE to failure, or when high occlusive pressures are used; 5) the metabolic stress evident during exercise with BFR is situationally adequate enough (during certain AE with BFR and LL-BFR RE taken to repetition failure; albeit evidently less stressful than both HIAE and HLRE) to reach a threshold necessary to promote an oxidative phenotype as seen with endurance exercise; and 6) the molecular and cellular physiological responses to exercise with BFR is unique,
but LL-BFR RE is relatively resemblant of HLRE, and the hypertrophic response to AE with BFR is probably consequential to oxidative stress.

From a practical standpoint, these findings can be further summarized and reorientated toward exercise sessions with the goal of muscle hypertrophy. A profitable BFR hypertrophy-based program ideally involves LL-BFR RE, with training conducted to the commonly implemented set and repetition scheme of 4 × 30-15-15-15. This regimen is particularly valuable in untrained individuals, as it is somewhat clearer that training to failure likely induces a minimal yet meaningful degree of muscle fiber stress. If RE is not possible, LIAE with BFR (cycling, walking) appears to be a valuable substitute, though the benefits are likely most conferred in untrained participants. A novelty of this review is the inclusion of studies evaluating WBV. Although only four included studies (Aguayo et al., 2016; Cai et al., 2018; Centner et al., 2019; Item et al., 2013) assessed some form of WBV, the collective findings show that BFR enhances metabolic and oxidative stress, as well as the acute satellite cell response during this exercise modality. This points to WBV as an additional low-risk modality for clinical use.

These conclusions provide value in that BFR exercise is most useful in untrained populations as trained populations would benefit more from traditional HLRE when the goal is muscle hypertrophy. Similarly, AE with BFR is inferior to traditional HIAE when the goal is endurance related adaptations, shown through acute molecular and cellular responses indicating metabolic stress (Smiles et al., 2017), which is to be expected. However, the use of BFR in trained participants likely permits more flexibility in a training program as stress responses in trained muscle are diminished. Specifically, BFR
enhances—in protocol and/or participant contingent manner—various physiological responses to HIAE (Christiansen et al., 2018), HLRE with WBV (Item et al., 2013), and HLRE (Cook et al., 2014). Therefore, use of BFR in trained individuals when combined with high-intensity exercise may confer additional benefits. Nevertheless, this method is, from a logical and evident standpoint, most profitable with low-intensity exercise in untrained, musculoskeletal compromised populations.

References


Amani-Shalamzari, S., Farhni, F., Rajabi, H., Abbasi, A., Sarikhani, A., Paton, C., Bayati, M., Berdejo-del-Fresno, D., Rosemann, T., Nikolaidis, P. T., & Knechtle,
https://doi.org/10.3389/fphys.2019.00614


https://doi.org/10.1097/PHM.0b013e3181951fc5


https://doi.org/10.1083/jcb.137.5.1057


https://doi.org/10.1084/jem.20070075


https://doi.org/10.1172/JCI119282


https://doi.org/10.1096/fj.02-0367com


https://doi.org/https://doi.org/10.1016/j.scispo.2020.03.006


Muscle Regeneration. *Front Physiol, 10*, 914.

https://doi.org/10.3389/fphys.2019.00914


https://doi.org/10.1111/apha.13045


https://doi.org/10.1152/japplphysiol.01298.2006


https://doi.org/10.1249/mss.0000000000000970


signaling and IGF-I-induced myocyte hypertrophy in C2C12 myocytes.


Kefaloyianni, E., Gaitanaki, C., & Beis, I. (2006). ERK1/2 and p38-MAPK signalling pathways, through MSK1, are involved in NF-kappaB transactivation during
oxidative stress in skeletal myoblasts. *Cell Signal, 18*(12), 2238-2251.
https://doi.org/10.1016/j.cellsig.2006.05.004


https://doi.org/10.6065/apem.2017.22.3.145

https://doi.org/10.1139/apnm-2015-0464


https://doi.org/10.3390/ijerph16244897


https://doi.org/10.1038/s41467-017-00520-9

https://doi.org/10.1519/JSC.00000000000003372


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skeletal muscle regeneration. *Cell Metab, 18*(2), 251-264.
https://doi.org/10.1016/j.cmet.2013.06.017

https://doi.org/10.1155/2012/973927


https://doi.org/10.1080/02640414.2017.1283430


https://doi.org/10.1111/apha.12243


https://doi.org/10.1177/0003319710375942


https://doi.org/10.1038/s41598-017-07182-z


https://doi.org/10.1016/j.cmet.2016.09.010


https://doi.org/10.1080/02701367.2019.1699234


https://doi.org/10.1016/j.molcel.2015.01.017


https://doi.org/10.1123/ijsnem.21.6.462

https://doi.org/10.1152/ajpregu.00545.2004

https://doi.org/10.1042/BJ20082055

https://doi.org/10.3389/fphys.2019.00929

https://doi.org/10.1016/j.mce.2006.11.012


https://doi.org/10.3390/ijms19113649


https://doi.org/10.4049/jimmunol.1101267


https://doi.org/10.1152/japplphysiol.00685.2018


upstream kinases is required for activity in mammalian cells. *Biochem J, 474*(17), 3059-3073. https://doi.org/10.1042/BCJ20170458


Yanagisawa, O., & Fukutani, A. (2018). Effects of low-load resistance exercise with blood flow restriction on intramuscular hemodynamics, oxygenation level and
https://doi.org/10.23736/S0022-4707.17.07463-1


https://doi.org/10.1152/japplphysiol.01185.2004

https://doi.org/10.1556/APhysiol.100.2013.4.6


