

What phenomena do occur in blood flow-restricted muscle?

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Oxygen is an essential molecule for all cellular activities including growth. Either excessive or deficient oxygen supply to cells induces the various responses of the cells. In the field of pathophysiology, effects of blood flow restriction on various organs have been studied for the past ~130 years. Subsequently, the roles of oxygen at subcellular level have been studied in vitro. Although a number of studies show that a low-intensity exercise (20~50% of one repetition maximum) with a moderate tourniquet restriction of blood flow results in increases in muscular strength and size, the mechanisms for this muscular adaptation remain unclear. In particular, it is uncertain whether the low-intensity exercise with blood flow restriction using a tourniquet causes the hypoxia or hyperoxia in the muscle, and then what signals leading to muscular hypertrophy are activated inside and/ or outside the cells. Also, it is not well understood what side effects occur in addition to conferring the benefits of strength gains. The review summarizes recent studies on the muscular adaptations to oxygen environment and discusses the mechanisms that may be involved in the resistance exercise with restricted blood flow.

Key words: oxygen, restriction of blood flow, skeletal muscle

INTRODUCTION

Skeletal muscle is sensitive to mechanical stress. It has been demonstrated that a high-intensity resistance training (>65% of one-repetition maximum, 1RM) causes increases in muscular size and strength [McDonagh et al., 1984]. On the other hand, exercise with a low-intensity (<65% of 1RM) primarily induces an improvement of muscular endurance capacity without considerable increases in muscular size and strength [McDonagh et al., 1984].

Muscular atrophy has been observed as a result of reduced physical activity and prolonged bed rest [LeBlanc et al., 1988]. However, high-intensity resistance training during the bed rest has been known to be a countermeasure against muscular atrophy and weakening.

These facts suggest that intense mechanical stress plays an important role in the maintenance and/ or the increase of muscular size and strength. Recently, however, it has been reported that a low-intensity resistance training (20~50% of 1RM) combined with a tourniquet restriction of blood flow causes increases in muscular strength and size [Shinohara et al., 1998; Takarada et al., 2000b; Abe et al., 2005]. These effects may involve, at least partially, increased muscle-fiber recruitment, and enhanced endocrine responses [Moritani et al., 1992; Takarada et al., 2000a].

Although the effects of blood flow restriction on skeletal muscle have been reported in the field of exercise physiology from 1960's, the field of medicine has reported the effects of blood flow restriction on

the physiological and anatomical status of various organs, including skeletal muscle from 1880's.

The purpose of this review is to summarize the available information about the mechanisms of muscular adaptations to blood flow restriction.

HISTORY

What has been studied in the medicine?

It is presumable that Volkmann's report in 1881 about ischemic contracture concomitant with fracture was the first publication of a complication related to blood flow restriction [Volkman, 1881]. Following the report, many studies have reported other status related to blood flow restriction, e.g. muscle edema and swelling [Jepson, 1926; Dennis, 1945]. In 1960, Haimovici pointed out the risk of arterial revascularization of ischemic extremities [Haimovici, 1960]. By the present, it has called attention to the ischemic/ reperfusion problem as well as the ischemia-induced problem.

It has been well known that the absolute occlusion of both venous and arterial veins (ischemia) induces serious damage to various tissues. In particular, skeletal muscle is vulnerable to ischemia. The critical tolerant time against ischemia in various tissues is as following: muscle, up to 4 h; nerve, up to 8 h; fat, up to 13 h; skin, up to 24 h; and bone, up to 4 days at normothermia [Steinau, 1988]. Actually, our previous study has shown that 1 day after the absolute blood flow occlusion for 30 min, some necrotic fibers are observed in the rat hindlimb

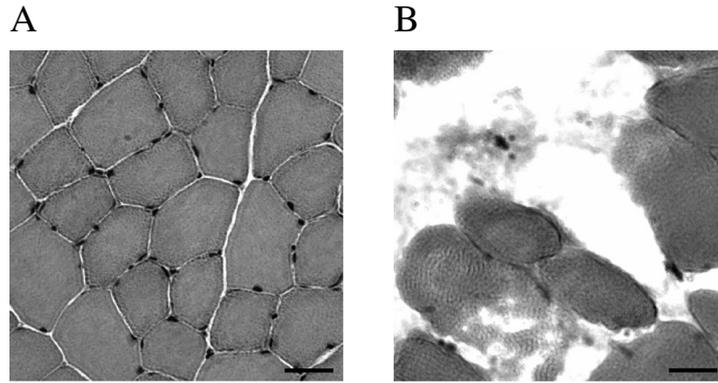


Figure 1. Cross-sections of plantaris muscle stained with hematoxylin/ eosin. A, sham-operated; B, both veins and arteries were occluded for 30min. Bars, 50 μ m (Kawada et al., 2005).

muscle (Figs. 1) [Kawada et al., 2005].

Although blood supply is important for the recovery from an ischemic injury in organs, reperfusion produces highly reactive substances derived from oxygen molecules, which induce inflammation through an activation of leukocytes [Smith et al., 1991; Menger et al., 1992; Gute et al., 1995]. Since leukocytes accumulation is observed in muscle after ischemia/ reperfusion, the leukocyte infiltration has been thought to contribute, at least partially, to the pathogenesis of reperfusion injury [Carden et al., 1989; Zimmerman et al., 1990]. In addition to the leukocytes, the enzyme xanthine oxidase (XO), arachidonic acid, and mitochondrial electron transport chain are known as potential biologic sources of cytotoxic oxygen species. Both in vivo and in vitro investigations have shown that SOD, a superoxide radical scavenging enzyme, and allopurinol or oxypurinol, XO inhibitors attenuate ischemia/ reperfusion-induced microvascular and parenchymal cell dysfunction in a wide variety of tissues [Korthuis et al., 1985; Lee et al., 1987; Grisham et al., 1988].

What has been studied in the exercise physiology?

In the physiology, including exercise physiology, effects of blood flow restriction/ reperfusion on muscular metabolism, strength, and size have been studied. In 1962, Fales and colleagues showed the different effects between partial venous occlusion and complete arterial occlusion in dog gastrocnemius/ plantaris muscle complex [Fales et al., 1962]. They demonstrated that partial venous occlusion causes decreases in the oxygen consumption in the muscles due to reduced blood flow, but on release of partial venous occlusion, little hyperemia and repayment of oxygen debt occur. On the other hand, after complete arterial occlusion, hyperemia and repayment of the oxygen debt incurred during the occlusion. These data have suggested that, when the arterial occlusion

is released, blood rushes into a bed in which resistance is reduced, because of the arteriolar dilatation distal to the occluded portion are appeared when the artery is occluded. On the other hand, such a reactive hyperemia was not observed in venous blood flow restriction. It needs to be mentioned that, even though complete occlusion of the isolated femoral artery causes a complete restriction of blood flow transiently, the flow level returns to about 15~20% of the resting level due to collateral circulation. Therefore, the complete arterial occlusion in their study may result in partial blood restriction. In 1976, Chiu and colleagues demonstrated the elevation of creatine phosphokinase (CPK) concentration in serum after the ischemia/ reperfusion with a pneumatic tourniquet [Chiu et al., 1976]. In 1979, Larsson and Hultman demonstrated moderate decreases in muscle ATP and phosphorylcreatine (PC) and increases in muscle ADP and AMP, and both muscle and blood lactate concentrations during occlusion [Larsson et al., 1979].

Moritani and colleagues have demonstrated increased mean motor unit (MU) spike amplitude and frequency, and surface electromyogram spectra parameters during low-intensity (20% of 1RM) muscle contractions with restriction of blood flow as compared to unhindered muscle contraction [Moritani et al., 1992]. This is likely the first report on the effects of blood flow restriction using a tourniquet on physiological properties of muscle in humans. At this point, a question is raised: Does a low-intensity training regimen with blood flow restriction cause increases in muscular strength and size? Training at 40% of maximal voluntary contraction (MVC) combined with tourniquet blood restriction has been shown to cause a significant increase in the muscular strength in two weeks as compared to unhindered muscle [Shinohara et al., 1998]. Another research group has also reported that a 16-wk training at

30~50% of 1RM with tourniquet blood flow restriction increased both muscular strength and size as did a high-intensity (50~80% of 1RM) training [Takarada et al., 2000b].

MECHANISMS FOR THE INCREASES IN MUSCULAR STRENGTH AND SIZE

Neuromuscular responses

It is important to activate type II muscle fibers during exercise training for muscular hypertrophy, because these fibers have a larger capacity for hypertrophy than that of type I fibers. Also, it has been shown that high-intensity exercise training recruits large motor units (MU) and their associated type II muscle fibers, according to the 'size principle' [Henneman et al., 1965]. However, at the present, it has been known that the size principle is not applied to certain conditions, such as 'eccentric' contraction in which the contracting muscle is forcibly stretched, and contraction under blood flow-restricted condition [Nardone et al., 1988; Moritani et al., 1992]. Moritani and colleagues have demonstrated that significant increases in MU firing rate and MU spike amplitude during repeated muscle contractions at 20% 1RM with blood flow restriction [Moritani et al., 1992]. Moore and colleagues have also shown that training at 50% 1RM with blood flow restriction is enough to increase in isometric torque, and the training causes an increase in post-activation potentiation (PAP) which likely enhances the responsiveness of muscle to calcium [Moore et al., 2004]. It has been demonstrated that during sustained muscle contractions in a low-intensity exercise training (15-20% of maximum voluntary contraction, MVC), blood flow is restricted and type II muscle fibers are depleted of glycogen [Vollestand et al., 1984]. Although the large force or high speed of muscle contraction is required to recruit high threshold MUs during exercise, oxygen supply to the active muscle may also affect the recruitment of large MU. Therefore, blood flow restriction may induce neuromuscular adaptations of active muscle, such as alteration of MU firing and recruitment patterns, which would result in increased muscular strength.

Metabolic changes

It has been shown that the rate of phosphocreatine (PCr) breakdown in the exercised muscle is larger during exercise with blood flow restriction when compared to that without restriction of blood flow, whereas the concentration of plasma lactate is higher after the exercise with blood flow restriction than after that without restriction of blood flow [Yoshida et al., 1997; Takarada et al., 2000a]. It has been shown that, after the period of exercise training, the intramuscular concentration of glycogen is greater in the muscle exercised with blood flow restriction than

in the muscle exercised without blood flow restriction, whereas the adenosine 5'-triphosphate (ATP) concentration is lower [Brugomaster et al., 2003]. It is well known that prolonged contractile activity can enhance muscle glycogen storage. It has also been shown that hypoxia activates muscle glucose transport by increasing the glucose transporter-4 (GLUT-4) translocation [Cartee et al., 1991]. In addition, it has been reported that high-intensity sprint training attenuates the magnitude of ATP depletion during exercise and reduces the resting muscle ATP content despite improving sprint performance [Stathis et al., 1994]. Although the precise mechanism for this muscular adaptation is unknown, it is most likely that high-intensity exercise training would change the balance between ATP hydrolysis and resynthesis. These results suggest that exercise training with blood flow restriction makes the metabolism more anaerobic similar to high-intensity exercise training.

Endocrine responses

It has been reported that concentrations of plasma growth hormone (GH), norepinephrine (NE) and insulin-like growth factor-1 (IGF-1) increase after low-intensity exercise training with blood flow restriction [Takarada et al., 2000a; Abe et al., 2005]. A large body of studies have reported that high-intensity exercise training regimens aiming muscular hypertrophy stimulate the secretions of hormones and growth factors [Kraemer et al., 1998; Borst et al., 2001]. Kraemer and colleagues have shown that high-intensity exercise training (10 repetitions maximum, 1-min rest between sets) causes an increase in plasma GH [Kraemer et al., 1990]. Goto and colleagues have pointed out the importance of metabolic subproduct accumulations for increasing muscular strength and size [Goto et al., 2005]. They compared the effects of two exercise regimens with same relative intensity and volume: a regimen without or with rest period inserted within a set. The regimen without rest period caused larger increases in the concentrations of blood lactate, serum GH, epinephrine and NE, and resulted in larger increases in muscular strength and size than after the regimen with a rest period.

Transgenic animals in which GH is overexpressed show abnormal muscularity [Dudley et al., 1987]. The circulating GH stimulates synthesis and secretion of IGF-1 from the liver and the skeletal muscle [Florini et al., 1996]. IGF-1 is one of the critical growth mediators of muscle. Overexpression of IGF-1 with virus mediated IGF-1 gene transfer has been shown to cause muscular hypertrophy [Barton et al., 1998]. However, the viral mediated overexpression of IGF-1 in muscles does not cause an increase in plasma IGF-1 level. Although the elevated

Table 1. Effects of low-intensity exercise training with blood flow restriction on physiological parameters. ↑, significant increase as compared to value of baseline or control group. ↓, significant decrease as compared to control group.

Physiological parameters	Changes
acute responses	
mean motor unit spike amplitude and frequency	↑
plasma lactate concentration	↑
plasma growth hormone concentration	↑
plasma IGF-1 concentration	↑
plasma norepinephrine concentration	↑
adaptations	
muscular size	↑
muscular strength	↑
muscle glycogen concentration	↑
muscle ATP concentration	↓

concentration of circulating IGF-1 has been shown after the low-intensity exercise training with blood flow restriction, the effect of circulating IGF-1 on the muscular hypertrophy remains unclear.

One study has reported that resistance exercise suppresses the secretion of GH during the first half of sleep [Nindl et al., 2001]. Even if the secretion of GH increases temporarily by the low-intensity exercise training with blood flow restriction, the total amount of GH secreted per day may not necessarily increase. Therefore, for muscular hypertrophy, it may be important, to enhance the GH release immediately after the exercise training, in addition to increase total amount of GH secreted per day.

The characteristic responses to low-intensity exercise training with blood flow restriction are shown in table 1.

Hypoxia or hyperoxia?

It has been reported that the external pressure for vascular occlusion is to be between mean systolic and diastolic blood pressure to cause increases in muscular strength and size [Takarada et al., 2000a]. Under such conditions, it has been thought to suppress both the venous outflow from and arterial inflow to the muscle. Compelling support for the notion is provided by the observation that low-intensity exercise training with blood flow restriction causes an accumulation of blood lactate [Takarada et al.,

2000a]. Therefore, it has been considered that the low-intensity exercise training with blood flow restriction causes local hypoxia, which makes the muscle metabolism more anaerobic, and suppresses the lactate clearance from the muscle. However, both the venous outflow from and arterial inflow to the muscle would cause the pooling of blood around the exercising muscle. Therefore, it is also possible that the blood flow restriction leads to hyperoxia rather than hypoxia within and/ or around the muscle. Few studies have reported on the oxygenation status in the muscle during restriction of blood flow. A near-infrared spectroscopy (NIRS) has been used to measure in vivo oxygenation status non-invasively and continuously. With NIRS (NIRO-200, Hamamatsu Photonics, Hamamatsu, Japan), we investigated the muscle oxygenation during varied patterns of exercise (Fig. 2). Exercise at 70% of 1RM caused a decrease in the level of muscle oxygenation ($[\Delta\text{HbO}_2]-[\Delta\text{Hb}]$, where HbO_2 and Hb are oxygenated and deoxygenated hemoglobins, respectively) during the exercise. Muscle oxygenation returned towards its resting level during the rest period between sets of the exercise (30sec) and then exceeded the resting level immediately after the exercise. Exercise at 35% of 1RM combined with tourniquet blood flow restriction also showed a decrease in the level of oxygenation during the exercise, but the level did not return to the resting level during the rest period between sets of exercise. However, after the release of external pressure, the level of oxygenation went up beyond the resting level, and the overshoot was larger than after the exercise at 70% of 1RM without blood flow restriction. These data indicate that low-intensity exercise training with blood flow restriction results in hypoxia during the exercise whereas hyperoxia after the exercise. In addition, the extents of both hypoxia and hyperoxia are larger than those during and after high-intensity exercise. Therefore, the amplitude of changes from hypoxia to hyperoxia may play a part in the muscular hypertrophy.

Effects of hypoxia on cell

Hypoxia affects gene expression in various cells. It has been demonstrated that fibroblasts exposed to low oxygen tension up-regulate the $\alpha 1(I)$ procollagen gene concomitant with up-regulated TGF- $\beta 1$ [Siddiqui et al., 1996; Falanga et al., 2002]. And also, proliferative activity of fibroblasts is enhanced under the hypoxia [Siddiqui et al., 1996]. Vascular endothelial growth factor (VEGF) has an important role in vasculogenesis and angiogenesis [Ferrara, 1999]. Because high levels of VEGF are constitutively expressed in many tumors, angiogenesis may be required for growth of cells, including skeletal muscle fibers. In fact, the muscular

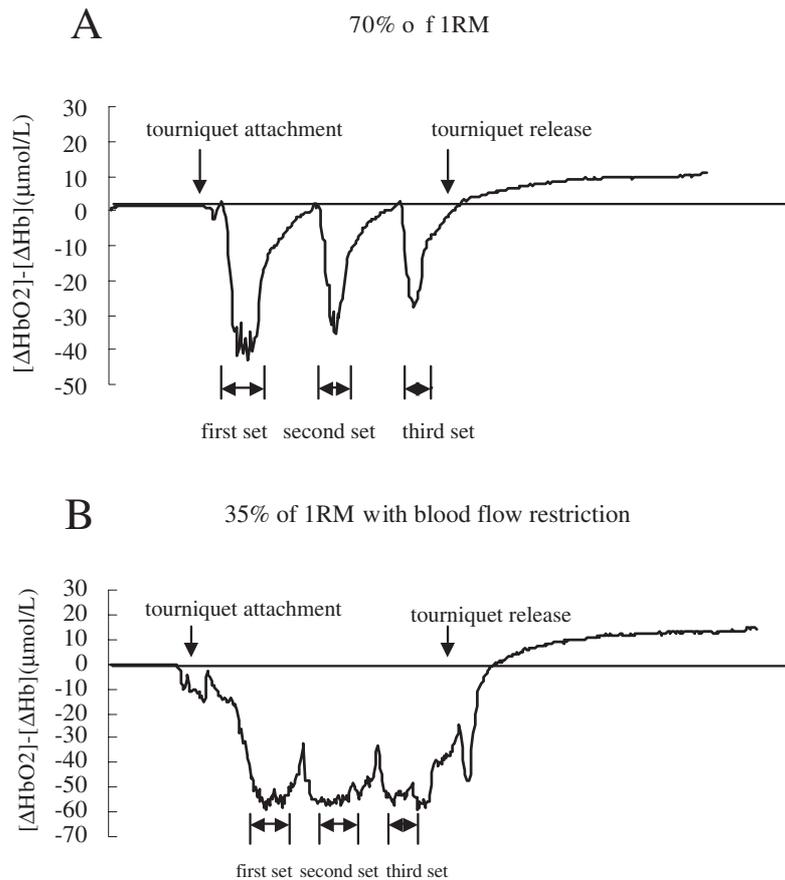


Figure 2. Oxygenation levels in *M. biceps brachii* during an arm curling exercise. A, exercise at 70% of 1RM consisted of 6-10 repetitions \times 3sets (10 repetitions for first set, 8 repetitions for second set, and 6 repetition for last set). B, exercise at 35% of 1RM with blood flow restriction consisted of 12-20 repetitions \times 3sets (20 repetitions for first set, 16 repetitions for second set, and 12 repetition for last set).

hypertrophy is usually associated with blood vessel neo-formation, in such that capillary density is either maintained or increased in the growing muscle, whereas muscular atrophy is associated with capillary loss [Reitsma, 1973; Plyley et al., 1998; Tynl et al., 2001]. It is well known that hypoxia enhances the expression of VEGF, which is activated by hypoxia-inducible factor 1 α (HIF-1 α) [Semenza, 2000]. In addition, active Akt, a serine-threonine protein kinase has been shown to cause a muscular hypertrophy and lead to a robust induction of VEGF expression, and the regulation is needed the HIF regulatory element on the VEGF gene [Semenza, 2000]. Akt is activated through the phosphatidylinositol 3-kinase (PI 3-kinase) pathway, which is regulated by IGF-1 [Datta et al., 1999; Takahashi et al., 2002]. Interestingly, both IGF-1 and hypoxia cause an increase in lactate, biochemical marker of myocyte hypertrophy [Semsarian et al., 1999]. Hypoxia and lactate, either alone or in combination, stimulate VEGF synthesis and release [Constant et al., 2000].

These data could be an explanation for the observations that angiogenesis is enhanced when lactate levels are elevated [Hunt et al., 1978]. Although the role of VEGF in the muscular hypertrophy is not fully understood, it may be one of the key factors in the muscular hypertrophy.

Effects of hyperoxia on cell

Hyperoxia leads to the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂) and hypochlorous acid (HOCl). ROS produced by hyperoxia is known to cause cell injury including lipid peroxidation, enzyme inactivation, and DNA damage [Cacciuttolo et al., 1993]. A large body of evidence has indicated that signaling pathways such as the mitogen activated protein kinases (MAP-Ks) may play important roles in regulating hyperoxic cell injury. The major subfamily members include extracellular signal-regulated kinase (ERK1/2), c-jun N-terminal protein kinase (JNK), and p38 kinase [Davis, 1993]. ERK1/2 is considered

to be a survival mediator against cell death, whereas JNK and p38 are related to the cell cycle arrest or apoptosis. However, it has also been reported that activation of ERK1/2 induces cell death in mouse macrophages and rat pheochromocytoma cells after hyperoxia [Petrache et al., 1999; Katoh et al., 1999]. Zhang and colleagues have also shown that hyperoxia-induced, murine lung epithelial cell death is attenuated by inhibiting ROS production or by inhibiting ERK1/2 activation prior to hyperoxia exposure [Zhang et al., 2003].

On the other hand, recent studies have given insights into possible mechanisms of cell growth under hyperoxia, although the effective data are still limited. It has been shown that hyperoxia enhances the proliferation rate of fibroblast in vitro [Hehenberger et al., 1997]. And also, hydroxylation of proline and lysine in procollagen is essential for stabilizing triple-helices of collagen fibrils, to which a large amount of oxygen is required.

It is presumable that signaling pathways and sensitivity to hyperoxia are different between cell types. This is also a subject for further study.

PERSPECTIVES FOR FUTURE STUDIES

At this time, the mechanisms of muscular hypertrophy after the low-intensity exercise training with blood flow restriction are not fully understood. In particular, it is still unknown which stimulation, hypoxia or hyperoxia is more important for a trigger of muscular hypertrophy. We recently developed a new animal experimental model, in which muscular hypertrophy was induced with chronic venous occlusion [Kawada et al., 2005]. Rats subjected to occlusion of veins from the hindlimb muscles caused an increase in hindlimb muscular size in 14 days. In this hypertrophied muscle, the expression of heat shock protein-72 (HSP-72), nitric oxide synthase-1 (NOS-1) mRNA increased, whereas that of myostatin protein decreased as compared to sham-operated control group. It has been shown that exercise training causes an increase in muscle HSP-72 content in rodents and humans [Naito et al., 2001; Thompson et al., 2003]. Although the exact roles of HSP-72 in protein metabolism in skeletal muscle are not fully understood, it may stabilize both existing and newly synthesized proteins. An increased expression of NOS-1 within muscle fibers may also play an important role in muscular hypertrophy, because several recent studies have shown that nitric oxide (NO), produced by NOS, stimulates the muscle growth [Anderson, 2000; Tatsumi et al., 2002]. A NOS inhibitor N ω -nitro-L-arginine methyl ester hydrochloride suppresses the activation of muscle satellite cells, whereas a NO donor, sodium nitroprusside dehydrate, promotes their proliferation [Tatsumi et al., 2002]. NO has also been shown to

mediate the expression of vinculin and talin, which are cytoskeletal proteins responsible for force transmission in a variety of cells [Tidball et al., 1999]. In our study, NO content in the hypertrophied muscle tended to increase. On the other hand, the hypertrophied muscle was associated with the decrease in muscle myostatin content. Myostatin, a member of the transforming growth factor- β (TGF- β) superfamily, is a potent negative regulator of muscle growth [McPherron et al., 1997a], and its content within muscle has been shown to decrease in response to mechanical stress [Kawada et al., 2001]. As has been known, mutations of its gene result in overgrowth of musculature in mice, cattle and humans [McPherron et al., 1997a, b; Schuelke et al., 2004]. Although it is not clear that hyperoxia is essential for muscular hypertrophy at this point, hypoxia may more likely stimulate the muscular hypertrophy.

POSSIBLE RISKS

Because low-intensity exercise training with blood flow restriction produces less mechanical stress when compared to normal high-intensity exercise training, it has been applied to elderly people with low physical fitness and athletes in rehabilitation. However, there is a serious question: Is it safe for anyone? Since the mechanisms of muscular strength and size gained by blood flow restriction are not fully elucidated, there is a controversy about the safety of the training method. It has been shown that occlusion of blood vessels sometimes causes the microvascular occlusion (no-reflow phenomenon) after releasing blood flow restriction, and results in muscle cell necrosis [Harmon, 1948; Strock et al., 1969]. Menger and colleagues suggested that the no-reflow phenomenon is due to intravascular hemoconcentration and thrombosis, swelling of capillary endothelial cells, plugging of the capillaries by leukocytes, and increased extravascular tissue pressure caused by interstitial edema formation [Menger et al., 1992]. What ever population trains using blood flow restriction always needs to pay attention to the no-reflow phenomenon.

In addition, low-intensity exercise training with blood flow restriction causes larger increases in both systolic and diastolic blood pressure as compared to low-intensity exercise training without blood flow restriction [Takano et al., 2005]. It is thought that the cardiac muscle may be overloaded mechanically. We have observed that the rats subjected to chronic blood flow restriction of hindlimb muscles show an increase in cardiac muscle size (data not shown).

Along with side effects of the low-intensity exercise training on health, its effects on cardiovascular functions need further elucidation.

CONCLUSION

Our understanding of low-intensity exercise training with blood flow restriction has grown quickly over the last few years. Work conducted over the past decade suggests that low-intensity exercise training combined with moderate blood flow restriction activates neuromuscular and endocrine systems. Efforts to explore the mechanisms for the response to hypoxia or hyperoxia in organisms will undoubtedly provide new therapeutic strategies.

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