Abstract In recent years, many experiments have investigated the impact of dietary amino acid supplementation on improvements in muscle strength and endurance capacity. However, it is unclear whether this supplementation causes vascular remodelling in skeletal muscle. This review focused on the effects of L-arginine and/or L-ornithine supplementation on capillary growth in cardiac and skeletal muscles. Although the chronic administration of 4% L-arginine did not improve capillarization in rat cardiac and hind-leg muscles, it facilitated exercise-induced capillary growth via vascular endothelial growth factor (VEGF) protein upregulation. In middle-aged rats, moderate intensity endurance training for 6 weeks did not cause capillary growth, whereas training with L-arginine supplementation led to an improvement in capillarization in the hind-leg muscle and left ventricle by promoting VEGF and endothelial nitric oxide synthase (eNOS) protein expression. The administration of L-arginine and L-ornithine for 6 weeks caused a marked increase in capillarization in rat skeletal muscle via the downregulation of endostatin and upregulation of VEGF-R2 protein expression. Moreover, this supplementation facilitated exercise-induced improvements in capillarization in the hind-leg muscles via the downregulation of endostatin and upregulation of VEGF and eNOS protein levels. The evidence presented in this review indicates that L-arginine and/or L-ornithine administration may facilitate capillary growth and endurance exercise capacity. Furthermore, this supplementation may be a useful therapeutic intervention for ischemic-related diseases.

Keywords: L-arginine, angiogenesis, endurance training, L-ornithine, vascular endothelial growth factor

Introduction

In recent years, many experiments have investigated the impact of dietary amino acid supplementation on improvements in muscle strength and endurance capacity. However, it is unclear whether this supplementation causes vascular remodelling in skeletal muscle. Angiogenesis, the development of new capillaries, is induced by a number of naturally occurring growth factors, hormones, and cytokines. Among these factors, vascular endothelial growth factor (VEGF) is thought to be an important regulator of angiogenesis during exercise training, as well as during embryonic development, wound healing, and tumor growth.

VEGF expression is predominantly increased in response to tissue hypoxia. Recently, the regulation of VEGF gene expression induced by NO has been demonstrated in numerous cell types, including vascular smooth muscle cells (N(G)-nitro-L-arginine (N-NNA), inhibitor of NO synthase (NOS), abolished exercise-induced capillary angiogenesis in skeletal muscle. In cardiac muscle, capillary density and VEGF mRNA expression was significantly lower in neonatal endothelial NOS-deficient (eNOS−/−) mice than in neonatal wild-type mice. These findings demonstrate that endogenous NO plays a critical role in VEGF-mediated angiogenesis.

This review focused on the effects of L-arginine, a substrate for NOS, and L-ornithine, a substrate for polyamines and L-proline, supplementation on capillary growth in cardiac and skeletal muscles.

Effects of L-arginine supplementation

Supplementation with L-arginine, a semi-essential amino acid, has been reported to induce positive effects on aerobic exercise performance and muscle adaptation. Chronic L-arginine, a substrate for NOS, treatment enhanced exercise-induced endothelial NO synthesis and aerobic capacity. Moreover, L-arginine has been shown to stimulate growth hormone and insulin release. Since both growth hormone and insulin are known to stimulate VEGF expression, increases in these hor-
mone levels may contribute to VEGF expression during chronic L-arginine supplementation.

Although the administration of 4% L-arginine for 6 weeks did not improve capillarization in rat cardiac and hind-leg muscles, it facilitated exercise-induced capillary growth, which was classified as an increase in the capillary-to-fiber ratio, via VEGF protein upregulation (Fig. 1)23). The expression of VEGF mRNA was increased after a single acute bout of exercise5-24), whereas chronic exercise training attenuated its expression in response to acute exercise in human skeletal muscle25). The expression of VEGF protein at capillary sites in rat skeletal muscles was significantly increased on the 6th and 10th days of training26). This expression was still high after 5 weeks of training, but was not significantly different from that of sedentary controls27). After 6 weeks of training with L-arginine, VEGF protein levels were still markedly higher than control level23). Therefore, the synergistic effects of exercise and L-arginine supplementation can sustain VEGF expression for longer periods, thereby improving capillarization.

Mechanical factors during exercise, such as shear stress and tissue stretching, may stimulate the expression of VEGF28,29). Chronic treatment with prazosin, a selective alpha1-adrenergic receptor antagonist, caused a 4-fold increase in shear stress and increased VEGF protein expression in rat skeletal muscle29). A recent study showed that shear stress activated the VEGF-R2 pathway, independent of VEGF29). The oral administration of 2.25% L-arginine solution for 8 weeks led to arteriolar enlargement and increased blood flow in the cerebrum of stroke-prone spontaneously hypertensive rats31). L-Arginine supplementation for 3 weeks enhanced acetylcholine (Ach)-induced vasodilation in the radial arteries of patients with severe chronic heart failure, and this supplementation with handgrip exercise produced an additive increase in vasodilation32). Although the infusion of L-arginine increased exercise-induced hyperemia during the handgrip exercise in patients with heart failure, it did not affect exercise hyperemia in control subjects33). Thus, it is unlikely that L-arginine supplementation facilitated flow-induced VEGF expression during exercise in individuals without cardiovascular disorders.

The possibility has been investigated that blood vessel formation in adults relies on growth from pre-existing capillaries. Circulating endothelial progenitor cells (EPCs) and resident EPCs, located in local tissues, have been identified as participants in microvascular remodeling34,35). While the mechanisms as to how EPCs contribute to exercise-induced angiogenesis in skeletal muscle are not known, acute exercise36) and chronic exercise training37) markedly increased circulating EPCs. The oral administration of 6% L-arginine solution for 4 weeks did not increase serum EPCs in mice, whereas L-arginine supplementation with physical training by swimming markedly enhanced EPC numbers and serum VEGF levels above that of training alone38). Therefore, it is possible that L-arginine supplementation may facilitate exercise-induced capillary growth via increased EPCs.

In aged rats, a recent study showed that endurance running at maximum tolerated speeds caused marked capillary growth, and that an increase in VEGF gene expression in response to acute exercise was not diminished by aging39). However, endurance training failed to cause capillary growth, which was classified as no increase in the capillary-to-fiber ratio, in middle-aged40) and old rats41).

Fig. 1  The effects of L-arginine treatment on capillarization in the left ventricle23). (A) Non-treated sedentary group. (B) Trained group. (C) L-arginine-treated group. (D) L-arginine-treated trained group. All images are at the same magnification.
when the intensity of training was moderate. In middle-aged rats, moderate intensity endurance training for 6 weeks did not cause capillary growth, whereas training with L-arginine supplementation caused marked exercise-induced capillary growth in the hind-leg muscle and heart by promoting VEGF and eNOS protein expression (Fig. 2)\(^42\). Thus, L-arginine supplementation may possibly improve exercise-induced microvascular remodelling in the elderly.

**Effects of L-arginine and L-ornithine supplementation**

The administration of L-arginine and L-ornithine for 6 weeks itself caused a marked increase in capillarization in rat skeletal muscle via the downregulation of endostatin and upregulation of VEGF-R2 protein expression\(^43\). Furthermore, in this study, this supplementation facilitated exercise-induced improvements in capillarization in the hind-leg muscles via the downregulation of endostatin and upregulation of VEGF and eNOS protein levels.

While L-arginine is metabolized to NO, it is catalyzed by arginase to produce L-ornithine. L-Ornithine is converted to putrescine by ornithine decarboxylase (ODC), after which putrescine is converted to spermidine and spermine (Fig. 3). These polyamines are known to be required for mammalian cell growth\(^44,45\). The overexpression of ODC may have enhanced endothelial cell proliferation by suppressing endostatin, a carboxy-terminal fragment of type XVIII collagen, which inhibits endothelial proliferation, angiogenesis, and tumour growth. NOS, NO synthase; OAT, ornithine aminotransferase; VEGF, vascular endothelial growth factor.

![Fig. 2](image-url) The effects of L-arginine treatment on capillarization in the soleus muscle of middle-aged rats\(^42\). (A) Non-treated sedentary group. (B) Trained group. (C) L-arginine-treated trained group. All images are at the same magnification.

![Fig. 3](image-url) Influences of L-arginine and L-ornithine supplementation on capillary growth. While L-arginine is metabolized to NO, it is catalyzed by arginase to produce L-ornithine. L-Ornithine is converted to putrescine by ornithine decarboxylase (ODC), after which putrescine is converted to spermidine and spermine. These polyamines are known to be required for mammalian cell growth. Overexpression of ODC enhanced endothelial cell proliferation possibly by suppressing endostatin, a carboxy-terminal fragment of type XVIII collagen, which inhibits endothelial proliferation, angiogenesis, and tumour growth. NOS, NO synthase; OAT, ornithine aminotransferase; VEGF, vascular endothelial growth factor.
fragment of type XVIII collagen, which inhibits endothelial proliferation, angiogenesis, and tumour growth. Endostatin was shown to influence the VEGF signaling pathway by the dephosphorylation of the ERK1/2-kinase in endothelial cells. Endostatin can also bind directly to VEGF-R1, -R2, and -R3 and thereby prevent the association of VEGF with their receptors. L-Ornithine is also converted to L-proline (Fig. 3), which is essential for the synthesis of collagen, by ornithine aminotransferase in vascular smooth muscle cells (SMC) and endothelial cells. The application of a cyclic stretch to SMC increased L-arginine uptake, L-proline production, and collagen synthesis. Thus, increased mechanical forces to the SMC by muscle contraction in conjunction with high L-arginine bioavailability may facilitate collagen synthesis around microvascular beds, and may also help to stabilize proliferating cells, thereby promoting angiogenesis.

Conclusion

Amino acid supplementation has been widely studied not only as ergogenic aids for athletes, but also as therapeutic interventions for diseases. The evidence presented here indicates that L-arginine and/or L-ornithine administration may facilitate capillary growth and endurance exercise capacity. Furthermore, this supplementation may be a useful therapeutic intervention for ischemic diseases, such as heart failure and intermittent claudication.

References

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