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Original article

Microcirculatory effects of L-arginine during acute anaerobic exercise in healthy men: A pilot study

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Abstract

Background/Objective: We hypothesized that L-arginine supplementation increases sublingual capillary perfusion during acute anaerobic exercise.

Methods: In a double-blind randomized study, 20 healthy men were randomly assigned to an L-arginine group or a placebo group. Both groups performed a standard 60-second duration BOSCO jumping test. Before the exercise, immediately after, and 30 minutes after exercise, systemic hemodynamic parameters were recorded. Sublingual evaluation of microcirculation using sidestream dark field (SDF) videomicroscopy was also carried out.

Results: There were no differences in mean arterial blood pressure and cardiac output between the placebo and L-arginine groups immediately after exercise and at 30 minutes after exercise. Both groups had no changes in the microvascular flow index and proportion of perfused vessels of small vessels over the testing period. We observed significantly higher functional capillary density [14.1 (12.5 − 16.0) vs. 11.7 (10.9 − 12.9) 1/mm, $p = 0.021$] and total vessel density of small vessels [27.8 (24.4 − 29.2) vs. 23.0 (21.6 − 24.2) mm/mm^2, $p = 0.041$] in the L-arginine group compared with the placebo group immediately after exercise, but after 30 minutes these differences had disappeared.

Conclusion: Our findings show that supplementation with L-arginine may cause additional effects on the acute anaerobic exercise-induced transient increase in capillary density in the sublingual mucosa of untrained men.

Keywords: Anaerobic exercise; L-arginine; Microcirculation

Introduction

Oxygen delivery through microcirculation depends on the convective properties of red blood cells (flow) and the oxygen diffusion distance between capillaries and cells, which reflects capillary density.

Theoretically, vasodilatation should be able to increase capillary density. Oral arginine supplementation, as a substrate for nitric oxide (NO) synthase, has the potential effect of increasing endothelium-dependent vasodilation in healthy subjects[1] and in patients with hypercholesterolemia,[2] essential hypertension,[3] coronary artery disease[4] and chronic heart failure.[5] Also, studies have demonstrated that oral arginine supplementation could improve exercise-induced vasodilation in subjects

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with various cardiopulmonary diseases.[6,7] A secondary effect of increased microcirculatory perfusion may be reduced exercise-induced increase in plasma metabolites, such as lactate and ammonia,[8] which would promote greater ability of organs to recover. It has been established that regular exercise training increases basal NO production[9] and probably maximizes capillary density. This may explain why some studies did not show any effect of arginine supplementation on plasma NO, lactate, and ammonia concentrations and performance during anaerobic tests in trained male athletes.[10] Therefore, we chose untrained men. Van Wijck and colleagues[11] used sidestream dark field (SDF) imaging to demonstrate that intake of L-citrulline, a precursor of arginine, by recreationally active men resulted in an increased number of perfused small sublingual vessels compared to placebo after cycling for 60 minutes at 70% of their maximum workload. However, it is not clear whether shorter duration and high intensity exercise are associated with changes in sublingual microcirculation in untrained men.

Anaerobic exercise is associated with situations in which oxygen supply to tissues may be inadequate in healthy humans.[12] Also, during intense exercise, blood flow is redistributed away from the splanchnic viscera toward working muscles, the cardiopulmonary system and the skin.[13] This physiological phenomenon of reduced splanchnic blood flow during intense exercise can cause gut injury with hemorrhagic stool.[14] Thus, the sublingual region may be a part in which changes in microcirculatory perfusion is visible.

Here, we raise the question of whether short duration of acute anaerobic exercise together with short-term L-arginine supplemention are sufficient for modulation of microcirculation in untrained men.

In this study, we chose the SDF imaging method to measure microcirculation in sublingual mucosa because this method is a superior non-invasive technique for assessing flow in individual capillaries in humans at the bed side, which can evaluate only accessible thin mucosa, mostly sublingual mucosa.[15,16] Currently, functional evaluation of individual capillaries is not possible in human muscles at the bed side. Also, clinical studies have shown that alterations of sublingual microcirculation are associated with worse outcomes.[17,18]

Materials and methods

Each subject volunteered to participate in the study after being informed of the purpose, experimental procedures, and known risks of the study. They each read and signed a written informed consent form that was consistent with the principles outlined in the Declaration of Helsinki. The local ethics committee approved this study.

Twenty apparently healthy males participated in the study. None of the participants were tobacco smokers or users of supplements. Also, none of them were athletes or regularly attended a fitness center. The men were instructed not to drink coffee or alcohol in the 24 hours before the experiment, and to arrive at the laboratory 4 hours after their meals. All experiments were performed at the same time of day to avoid the effects of circadian rhythm.

Experimental protocol

Participants were randomly assigned to an L-arginine group (10 men, body mass index of 23.7 kg/m^2) or a placebo group (10 men, body mass index of 23.1 kg/m^2). The L-arginine group was orally supplemented with 20 g of L-arginine in liquid form 45 minutes before the exercise. The placebo group was supplemented with a placebo drink of the same quantity, color, and taste 45 minutes before exercise. Both groups performed a standard 60-second duration jumping exercise (BOSCO test).

Before the exercise, immediately after, and 30 minutes after exercise, systemic hemodynamic parameters were recorded, such as mean arterial blood pressure (MAP), heart rate (HR), and cardiac output (CO) by impedance cardiography (BioZ, CardioDynamics, San Diego, CA, USA); and sublingual microcirculation was evaluated.

BOSCO anaerobic test

To reach the maximal mechanical power of the leg extensor muscle, the participants jumped on the platform continuously with maximal effort for the entire 60-second duration. Participants bent the knee to about 90 degrees to standardize the knee's angular displacement during the contact phase. During the test, an investigator watched the knee angle and instructed the participant to increase or decrease the depth of knee flexion as the test effort proceeded. Participants were required to keep their hands on their waists throughout the test to minimize the contribution of the upper body to the test performance. Verbal encouragement was provided by the investigators. To calculate the average mechanical power (W_a) during 60 seconds of jumps, the following formula was used: $W_a = (g \cdot T_f \cdot 60)/[4 \cdot n \cdot (60 - T_f)]$. Thus, to apply the formula, we only needed to know the sum of the flight time (T_f) recorded by the timer for each jumping performance and the number of jumps (n) executed during that work time period, where g = acceleration of gravity (9.81 m/s^2). Reliability of the BOSCO test has been reported at $r = 0.95$.[12] This test is used to determine explosive power. The jumping test results showed that anaerobic energy conversion reached a blood lactate concentration of around 8 mmol/L.[12]

Videomicroscopic measurements and analysis

Images of the sublingual microcirculation were obtained with SDF videomicroscopy (Microscan®; MicroVision Medical, Amsterdam, The Netherlands). After gentle removal of saliva and other secretions by isotonic saline-drenched gauze, the device was applied to the sublingual region, avoiding pressure artifacts by establishing a threshold-image. Sequences of 20 seconds from at least three areas were recorded on a hard disk using a personal computer and AVA v3.0 software (MicroVision Medical). Video clips were blindly analyzed offline by two investigators in random order to prevent coupling.

Each image was divided into four equal quadrants. Quantification of flow (no flow: 0; intermittent flow: 1; sluggish flow: 2; continuous flow: 3) was scored per quadrant, for each vessel diameter cohort (small: 10–20 μm; medium: 21–50 μm; large: 51–100 μm). The microvascular flow index (MFI) was calculated as the sum of each quadrant score divided by the number of quadrants in which the vessel type was visible. The final MFI was averaged over a minimum of 12 quadrants (three regions, four quadrants per region) derived from the overall flow impressions of all vessels with a particular range of diameter in a given quadrant.

Calculation of total vessel density (TVD) of small vessels was performed with the AVA software package (MicroVision Medical), as described and validated recently,[19] using a cut-off diameter for small vessels (mostly capillaries) of <20 μm. We defined the perfused vessel density of small vessels, an estimate of functional capillary density (FCD), and the proportion of perfused vessels (PPV) of small vessels in terms of the number and percentage of crossings with perfused small vessels per total length of three equidistant horizontal and three equidistant vertical lines. This method has been described elsewhere by de Backer et al. and is in accordance with reports of a round table conference.[20]

Statistics

Primary outcomes were sublingual FCD and TVD of small vessels. SPSS version 15.1 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. With respect to small sample size, data are presented as the median (25th–75th percentiles) and analyzed with non-parametric tests. A p value of <0.05 was considered significant.

Results

There were no significant differences between the placebo and L-arginine groups at baseline. During the BOSCO exercise test, participants in the placebo and L-arginine groups attained the same average power values [22.6 (16.9–28.8) vs. 20.1 (16.8–34.4) $W \cdot kg^{-1}$, $p = 0.935$]. In both groups, exercise significantly increased MAP, HR and CO (Table 1). Thirty minutes after exercise, unlike the placebo group, the L-arginine group's HR had returned to baseline. However, MAP after 30 minutes was decreased in both groups compared with baseline values. There were no differences in MAP and CO between placebo and L-arginine groups immediately after exercise and at 30 minutes after exercise. Immediately after exercise, a trend of increased CO was observed in the L-arginine group compared with that in the placebo group [12.0 (10.6–13.3) vs. 10.4 (9.5–11.4) L/min, $p = 0.065$].

Evaluation of sublingual microcirculation

The effects of exercise on sublingual microcirculation are presented in Table 2. Neither group showed changes in the MFI and PPV of small vessels over the testing period. We observed a significant increase in TVD of small vessels after exercise, but 30 minutes after exercise, the difference disappeared compared with baseline. After exercise, we observed significantly higher FCD [14.1 (12.5–16.0) vs. 11.7 (10.9–12.9) 1/mm, $p = 0.021$] and TVD of small vessels [27.8 (24.4–29.2) vs. 23.0 (21.6–24.2) mm/mm^2, $p = 0.041$] in the L-arginine group compared with the placebo group.

Digital microphotographs of microcirculation during exercise in the L-arginine group are presented in Figure 1.

Table 1
Systemic hemodynamic response to acute anaerobic exercise following supplementation with L-arginine and placebo.

	Placebo	L-Arginine	p
Age, y	22 (22–24)	23 (22–24)	0.626
Weight, kg	74.4 (69.9–85.9)	79.8 (73.2–87.9)	0.384
Height, cm	179.0 (175.0–184.5)	180.0 (176.0–191.5)	0.427
BMI, kg/m^2	23.1 (22.0–24.8)	23.7 (22.2–25.1)	0.496
Heart rate, beats/min			
Baseline	80 (76–86)	77 (71–89)	0.544
End-exercise	136 (120–144)*	136 (117–144)*	0.990
30 min after exercise	89 (82–108)*	85 (74–102)	0.364
MAP, mmHg			
Baseline	92 (87–97)	92 (86–99)	0.909
End-exercise	109 (90–121)*	114 (105–125)*	0.344
30 min after exercise	88 (80–91)*	89 (84–91)*	0.732
Cardiac output, L/min			
Baseline	6.9 (6.3–7.4)	7.1 (6.1–7.9)	0.791
End-exercise	10.4 (9.5–11.4)*	12.0 (10.6–13.3)*	0.065
30 min after exercise	6.6 (6.1–6.9)	7.4 (5.3–9.4)	0.677
SVRI, dynes·sec/cm^5/m^2			
Baseline	1830 (1555–2066)	1980(1661–2171)	0.436
End-exercise	1760 (1410–1910)*	1538 (1300–1875)*	0.497
30 min after exercise	1860 (1800–2020)	1788 (1443–2181)	0.631

Values are median (25th–75th).
Significantly different from baseline: *$p < 0.05$.
BMI = body mass index; MAP = mean arterial pressure; SVRI = systemic vascular resistance index.

Table 2
Microcirculatory response to acute anaerobic exercise following supplementation with L-arginine and placebo.

	Placebo	L-Arginine	p
MFI			
Baseline	3.00 (2.88–3.00)	2.92 (2.92–3.00)	0.435
End-exercise	3.00 (2.67–3.00)	2.92 (2.90–3.00)	0.871
30 min after exercise	3.00 (2.90–3.00)	2.96 (2.67–3.00)	0.430
PPV, %			
Baseline	98.6 (96.1–99.5)	97.5 (95.0–98.3)	0.112
End-exercise	98.2 (97.9–98.9)	97.9 (96.2–99.6)	0.820
30 min after exercise	98.7 (97.0–99.1)	98.1 (95.7–98.7)	0.186
FCD, 1/mm			
Baseline	11.6 (9.5–13.6)	12.8 (11.4–15.2)	0.199
End-exercise	11.7 (10.9–12.9)	14.1 (12.5–16.0)	0.021
30 min after exercise	11.5 (10.6–12.6)	14.3 (10.6–15.6)	0.104
TVD, mm/mm^2			
Baseline	19.5 (17.8–24.1)	24.1 (20.1–28.8)	0.112
End-exercise	23.0 (21.6–24.2)*	27.8 (24.4–29.2)*	0.041
30 min after exercise	22.5 (20.4–24.0)	26.6 (21.0–29.9)	0.096

Data are median (25th–75th).
Significantly different from baseline: *$p < 0.05$.
MFI = microvascular flow index of small vessels; PPV = percentage of perfused small vessels; FCD = functional capillary density; TVD = total vessel density of small vessels.

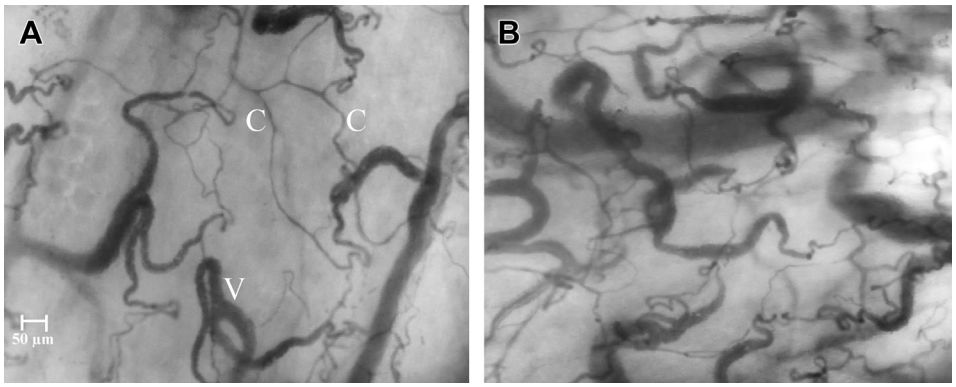

Fig. 1. Digital microphotographs of microcirculation before (A) and immediately after (B) exercise in the L-arginine group. Sidestream dark field videomicroscopy is based on the principle that emitted green light (wavelength 530 nm) is absorbed by the hemoglobin content in red blood cells. Thus, red blood cells are seen as black or gray bodies during imaging. The vessel walls are not visualized, so vessels can only be detected by the presence of red blood cells. C = capillaries; V = venules.

Discussion

In the present study, short duration of acute anaerobic exercise with or without L-arginine supplementation caused increase in total small vessel (mostly capillaries) density in the sublingual mucosa of untrained men. However, supplementation with L-arginine induced a more pronounced increase in capillary density. These effects were temporary and disappeared after exercise. To our knowledge, this is the first study evaluating the influence of short-term L-arginine supplementation on microcirculation in humans during acute anaerobic exercise.

Acute transient increase in total capillary density may be associated with the systemic effects of acute exercise, such as increased blood viscosity[21] and decreased red blood cell deformability.[22] Animal studies have demonstrated that increased blood viscosity may decrease vascular resistance and increase tissue perfusion by increasing wall shear stress,[23] which may induce NO production by endothelial cells to create vasodilatory compensation.[24]

There is currently ongoing debate challenging the phenomenon of recruitment of new capillaries. It is estimated that although 100% of capillaries in skeletal muscle at rest are perfused by plasma, only around 80% contain erythrocytes.[25] In our study, vessels were measured by the detection of flowing erythrocytes as distinct from the visualization of vessel walls; therefore, capillaries without erythrocytes would remain undetected at rest. With the influx of erythrocyte flow into these capillaries during exercise, they would then become visible, hence increasing capillary density. According to a new theory, erythrocyte flux in to capillaries is controlled by arterioles and not by precapillary sphincters.[25] Theoretically, according to Poiseuille's Law, vasodilatation and/or increased CO should be able to increase blood flow at the entrance of the capillaries and increase influx of red blood cells. In our study, we observed increased CO and decreased systemic vascular resistance immediately after exercise in both groups. However, the groups did not differ in accordance with the following parameters, but there was a trend for higher CO in the L-arginine group. Therefore, it is difficult to assign an importance of these two factors for pronounced effect of L-arginine on capillary density in sublingual mucosa. In contrast to our results, previous studies suggested that arginine supplementation could improve exercise-induced vasodilation in subjects with various cardiovascular diseases.[6,7]

Most studies have evaluated muscle microcirculation during aerobic exercise using intravital microscopy in animals or muscle capillary density in muscle biopsy in humans. Human studies have shown that endurance (aerobic) training increases capillary density in skeletal[26] and cardiac muscles.[27] It is estimated that muscle capillary density is lower in women than in men,[28] and is also lower in untrained than in trained subjects.[29] With training, capillary density increases more in women than in men.[30] To preserve the functional balance between metabolic demand and oxygen delivery, the skeletal and cardiac muscles increase capillary density and/or the capillary-to-fiber ratio. During anaerobic exercise, the body is exercising at such a rate that the blood stream cannot supply oxygen to muscles fast enough. This happens during high-intensity acute exercise and can be observed during football, basketball, rugby, or hockey. The BOSCO jumping test or the WINGATE treadmill test are often used for anaerobic testing. In agreement with the BOSCO test,[12] the concentration of blood lactate reached high values (approximately 8 mmol/L). This supports the notion about anaerobic energy conversion during the BOSCO test.

In our study, L-arginine supplementation induced a more pronounced increase in sublingual capillary density in untrained men. In line with our results, Van Wijck and colleagues[11] demonstrated that intake of L-citrulline, a precursor of arginine, resulted in an increased number of perfused small sublingual vessels compared with placebo after cycling for 60 minutes. In this study, the improvement in sublingual perfusion was in line with attenuated intestinal injury during exercise. Different from Van Wijck et al.,[11] we used shorter duration (60 seconds instead of 60 minutes) and a completely anaerobic exercise (BOSCO jumping test instead of cycling for 60 minutes at 70% of maximum workload). Also different from their study, we recorded our results of microcirculation according to density and flow parameters as recommended.[20] It is important to report the number of vessels per grid

length or image area unit, and not just the total number of vessels, because image stabilization during the process of analysis may reduce the image area.

Studies have suggested that arginine supplementation has the potential effect of increasing endothelium-dependent vasodilation and could improve exercise-induced NO production and vasodilation in people with low exercise capacity with various cardiovascular diseases.[1–6] Also, short-term oral arginine supplementation in the untrained population could reduce exercise-induced lactate and ammonia accumulation.[8,31] However, other studies have shown that short-term arginine supplementation has no effect on plasma NO, lactate, and ammonia concentrations in intermittent anaerobic tests in well-trained male athletes.[10]

These different results may be explained by the fact that regular exercise training increases basal NO production by stimulating endothelial NO synthase expression and phosphorylation so that it reaches higher concentrations than in the general population.[9] Also, increased muscle perfusion due to elevated NO production may improve muscle aerobic metabolism and less lactate accumulation.[8]

Animal studies have shown that L-arginine administration alone did not affect capillary density in the heart and hind-leg muscles in the young rat.[32] We also observed no significant changes in the L-arginine group compared with the placebo group during the 45 minutes after L-arginine ingestion before exercise. Supplementation with L-arginine, a precursor of NO, enhanced exercise-induced endothelial NO synthesis.[33] Exercise and supplementation with L-arginine both increase NO synthesis by skeletal muscle.[34] During adaptation to exercise, NO plays an important regulatory role by increasing blood flow to the muscles and modulating muscle contraction and glucose uptake.[35] NO is involved in controlling cellular respiration and may act as an antioxidant in some situations, such as in systemic or local hypoxia.[36,37] A variety of factors, such as catecholamine, acetylcholine, blood shear stress, and systemic or local hypoxia, may induce release of NO from the vascular endothelium.[36,38]

Additional effects on microcirculation during acute anaerobic exercise may be associated with systemic or local hypoxia, tissue stretching and cellular damage, reactive oxygen species (ROS) production, and cytokine expression. Systemic hypoxia significantly dilated arterioles, and increased blood flow, capillary perfusion, and ROS in hamster's cheek pouch.[36] The administration of NO donors is beneficial in preventing increases in ROS formation during early reperfusion characterized by low oxygen tension.[36,39] Interestingly, oxygen is partly supplied by arterioles during normoxia, whereas during hypoxia, capillaries appear to be the major suppliers of the oxygen delivery to the tissue.[36] The level of NO is crucial to maintaining vasodilation during hypoxemia to prevent vasoconstriction. Furthermore, after postischemic reperfusion, the no-reflow phenomenon was negligible after both L-arginine and NO donors, and capillary blood cell velocity was significantly higher than that observed in control animals.[40] Animal studies have shown that preconditioning with intermittent hypoxia can greatly reduce oxidative stress and stimulate NO-induced vasodilation during ischemia-reperfusion injury, thus controlling capillary perfusion.[41] However, in the present study, we did not measure blood gases or the above-mentioned pathophysiological processes.

We observed no changes in power between the groups during the 60 seconds of the BOSCO anaerobic test. Several studies have shown that arginine supplementation could increase exercise performance in patients with cardiovascular diseases.[6,7,42] However, other studies report no ergogenic effect of L-arginine supplementation on various aerobic and anaerobic tests in patients and healthy subjects. Olek et al.[43] demonstrated that a single 2-g oral dose of L-arginine given before three 30-second all-out supramaximal Wingate Anaerobic Tests had no effect on exercise performance compared to the placebo group. Liu et al.[10] evaluated the effect of L-arginine 6 g daily for 3 days on the performance of well-trained male judo athletes in an intermittent cycle ergometer anaerobic exercise test. Although plasma L-arginine levels increased, there were no effects on plasma nitrate or nitrite or on peak and average power in the exercise test.

Our study has some limitations, such as short observation time and relatively high L-arginine dose. To our knowledge, we are the first to demonstrate that microcirculation can quickly respond to oxygen demand in humans. Other studies have mostly evaluated 6 g of L-arginine supplementation. It has been reported that low oral doses ($\leq$20 g) of arginine are well tolerated, and adverse effects are rare in healthy humans.[44] We did not observe any side effects in our study. On the other hand, we tried to find the maximum possible effect of L-arginine supplementation on sublingual microcirculation.

In conclusion, in this pilot study, we were able to demonstrate that L-arginine supplementation may cause additional effects on acute anaerobic exercise-induced transient increase in capillary density in the sublingual mucosa of untrained men. The present results suggest that sublingual mucosa is a reasonable site for detection of microcirculatory changes at the bed side in healthy subjects.

Conflicts of interests

The authors declare that they have no competing interests.

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