L-Arginine enhances aerobic exercise capacity in association with augmented nitric oxide production

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L-Arginine enhances aerobic exercise capacity in association with augmented nitric oxide production. J Appl Physiol 90: 933–938, 2001.—We tested whether supplementation with L-arginine can augment aerobic capacity, particularly in conditions where endothelium-derived nitric oxide (EDNO) activity is reduced. Eight-week-old wild-type (E+) and apolipoprotein E-deficient mice (E−) were divided into six groups; two groups (LE+ and LE−) were given L-arginine (6% in drinking water), two were given D-arginine (DE+ and DE−), and two control groups (NE+ and NE−) received no arginine supplementation. At 12–16 wk of age, the mice were treadmill tested, and urine was collected after exercise for determination of EDNO production. NE− mice demonstrated a reduced aerobic capacity compared with NE+ controls [maximal oxygen uptake (VO2 max) of NE− = 110 ± 2 (SE) vs. NE+ = 122 ± 3 ml O2·min−1·kg−1, P < 0.001]. This decline in aerobic capacity was associated with a diminished postexercise urinary nitrate excretion. Mice given L-arginine demonstrated an increase in postexercise urinary nitrate excretion and aerobic capacity in both groups (VO2 max of LE+ = 120 ± 1 ml O2·min−1·kg−1, P < 0.05 vs. NE−; VO2 max of LE− = 133 ± 4 ml O2·min−1·kg−1, P < 0.01 vs. NE−). Mice administered D-arginine demonstrated an intermediate increase in aerobic capacity. We conclude that administration of L-arginine restores exercise-induced EDNO synthesis and normalizes aerobic capacity in hypercholesterolemic mice. In normal mice, L-arginine enhances exercise-induced EDNO synthesis and aerobic capacity.

oxygen uptake; vascular reactivity; hypercholesterolemia; apolipoprotein E knockout; D-arginine; endothelium-derived relaxing factor

SKELETAL MUSCLE ARTERIOLES vasodilate in response to exercise to augment nutrient and oxygen delivery to working muscles (29, 33). Since its discovery as an endogenous vasodilator, the role of endothelium-derived nitric oxide (EDNO) in mediating this vasodilatory response to exercise has been investigated with conflicting results reported (7, 11, 13, 20, 23, 34). Our laboratory recently reported that EDNO contributes to exercise hyperemia and is a determinant of aerobic capacity in exercising mice (20). In that study, exercise was associated with increased blood flow to the hindlimb muscles and an acute increase in urinary excretion of nitrogen oxides (used as a measure of EDNO production; Ref. 3) after exercise. Both of these effects were prevented by administration of the nitric oxide synthase (NOS) inhibitor N(G)-nitro-L-arginine. Furthermore, hypercholesterolemic mice manifest decreased excretion of urinary nitrates after exercise, reduced endothelial vasodilator function, and reduced aerobic capacity. These data suggest that conditions of reduced ENDO synthesis or activity (e.g., hypercholesterolemia) result in an inadequate exercise hyperemic response that is rate limiting to oxygen transport and exercise capacity. If so, L-arginine supplementation may improve exercise performance in these conditions.

The present study was performed to determine whether supplementation with L-arginine would prevent the decline in aerobic capacity observed in hypercholesterolemic mice. In pilot studies, we determined that the exercise capacity of wild-type (E+) and apolipoprotein E-deficient mice (E−) are the same at 8 wk of age when the cholesterol levels of both strains are low. After 8 wk of age, the cholesterol levels of E− mice rise rapidly to >1,000 mg/dl by 12 wk of age. At the same time, the principal measure of exercise capacity, maximal oxygen uptake (VO2 max), declines to about 85% of controls. This decline is associated with endothelial vasodilator dysfunction and reduced urinary nitrate excretion. The present study was designed with the intention of averting the impairment in aerobic capacity associated with hypercholesterolemia through chronic supplementation of L-arginine.

MATERIALS AND METHODS

Animals. Eight-week-old female E+ and E− C57BL/6J mice [Jackson Laboratories, Bar Harbor, ME, and Stanford Department of Comparative Medicine (DCM)] were entered into experimental protocols after a 1-wk period of acclimation in the housing facilities of the DCM. All mice were inspected before the study by the DCM veterinarian and monitored daily by DCM technicians and investigators. All experimental protocols were approved by the Administrative Panel on Animal Care.

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Laboratory Animal Care of Stanford University and were performed in accordance with the recommendations of the American Association for the Accreditation of Laboratory Animal Care. All mice were housed three to four per cage. They were maintained on a 12:12-h light-dark cycle and given unlimited access to food and water for the duration of the study. All mice were taught to run on a treadmill with shock-plate incentive (Exer-4 Treadmill, Columbus Instruments, Columbus, OH) but were otherwise confined to cages for the duration of the study.

The E− mice were generated from targeted disruption of the apolipoprotein E gene in the 129 embryonic stem cell line. Germ-line chimeras were mated and backcrossed for 10 generations with C57BL/6J wild-type mice (25).

**Experimental protocol.** Eight-week-old E− and E+ mice were divided into six groups (Table 1): two groups were supplemented with L-arginine (6% drinking water, LE−; n = 16 and LE+; n = 16); two were administered D-arginine (the optical isomer of L-arginine, which is not a considered a substrate for NOS, 6% drinking water, DE−; n = 8 and DE+; n = 8); and two received regular drinking water (NE−; n = 27 and NE+; n = 24). The mice were kept sedentary for 4–8 wk. At 12–16 wk of age, the mice were treadmill tested in random order by an investigator blinded to the identity of its group to measure indexes defining exercise capacity. Because this study was designed to determine the effect of chronic enhancement of EDNO production rather than an acute effect of arginine, all water bottles containing arginine were replaced with regular water 48 h before treadmill testing. Urine was collected after treadmill exercise for determination of urinary nitrogen oxides (NOx). Mice were killed after treadmill testing by overdose of methoxyflurane (Pitman-Moore, Mundelein, IL) inhalation anesthesia. Blood was collected at the time of death. These were immediately centrifuged at 4,000 rpm for 5 min, and the supernatant was collected, diluted 1:9 in distilled water, and stored at −80°C for measurement of NOx and creatinine.

NOx in the urine was measured with a commercially available chemiluminescence apparatus (model 2108, Dasibi, Glendale, CA) (30). Briefly, the samples (50 µl) were injected into boiling acidic vanadium (III) chloride. This technique utilizes acidic vanadium (III) chloride at 98°C to reduce both NO3− and NO2− to NO, which is then detected by the chemiluminescence apparatus after reacting with ozone. Signals from the detector were analyzed by computerized integration of curve areas. Standard curves for NaNO2/NaNO3 were linear over the range of 50 pM to 10 nM. Urine creatinine was measured by the modified method of Slot developed by Sigma Diagnostics (10).

**Hematology and biochemistry.** Blood samples were collected at the time of death. These were immediately centrifuged at 3,000 rpm for 15 min. The serum was separated and stored at −80°C until analysis. Total serum cholesterol was analyzed using the enzymatic method of Allain et al. (1) as developed by Sigma Diagnostics.

**Data analysis.** Four of the 99 mice died before study completion and were excluded from data analysis. Data are expressed as means ± SE. Comparisons of single means from multiple populations were made by one-factor univariate one-way ANOVA followed by Fisher’s protected least significant difference. A P value <0.05 was accepted as statistically significant.

**RESULTS**

**Effect of hypercholesterolemia on aerobic capacity and nitric oxide production.** The body weights of the NE− and NE+ mice were the same at the end of the study (Table 2). The cholesterol levels of the NE− mice were significantly elevated compared with NE+ mice (1,073 ± 80 vs. 157 ± 13 mg/100 ml serum, P < 0.0001). Similar to our previous report, both indexes of aerobic capacity of the 12- to 16-wk-old NE− mice were reduced compared with NE+ controls (Fig. 1) (19). VO2max of the NE− mice was 89% (P < 0.001) that of NE+ mice, and running distance was 77% (P < 0.005) that of NE+ mice. Our laboratory has previously reported that postexercise urinary nitrate levels are reduced in NE− compared with NE+ mice (20). Similarly, in the present study, postexercise urinary nitrate levels were less in the NE− compared with the NE+ mice (157 ± 25 vs. 433 ± 100 pmol/mg creatinine) (Fig. 2).

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**Table 1. Number of mice assigned to each treatment group**

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E+, wild type mice; E−, apolipoprotein E (apoE)-deficient mice; N, no supplementation; L, L-arginine supplementation; D, D-arginine administration.
Effect of arginine on aerobic capacity and nitric oxide production. Supplementation with L-arginine for 4–8 wk had no effect on body mass or cholesterol level in either strain. Mice in both strains supplemented with L-arginine demonstrated an increase in both indexes of exercise capacity. LE+ mice measured a 9% increase in VO2max (P < 0.05) and a 61% increase in running distance (P < 0.0001) compared with NE+. The improvement in aerobic capacity was accompanied by a fivefold increase in postexercise urinary nitrate excretion (156 ± 25 to 750 ± 145 pmol/mg creatinine, P < 0.05). Mice treated with D-arginine exhibited a trend toward an improvement in VO2max [7%, not significant (NS)] and an intermediate improvement in running distance (39%, P < .01). The D-arginine-treated mice also had a trend toward an increase in postexercise urinary nitrate excretion (61%, NS).

Normal mice also improved with administration of L-arginine; the LE+ mice had increased aerobic capacity as measured by an 8% increase in VO2max (P < 0.01) as well as a trend toward an increase in running distance compared with NE+. Postexercise urinary nitrate excretion also appeared to increase with L-arginine supplementation of the E+ strain (NS). DE+ mice exhibited little improvement in VO2max but did manifest a significantly greater running distance. DE+ mice also trended toward an increase in postexercise urinary nitrate excretion.

**DISCUSSION**

The salient findings of this study are that administration of L-arginine normalizes exercise-induced EDNO synthesis and aerobic exercise capacity in hypercholesterolemic mice and somewhat unexpectedly enhances exercise capacity in normal mice as well. This study confirms our previous finding that hypercholesterolemic mice manifest a reduced aerobic exercise capacity compared with normcholesterolemic controls (20). The effect observed with L-arginine, together with our previous observations of reduced limb blood flow, exercise capacity, and postexercise nitrogen oxide excretion in both hypercholesterolemic animals and animals administered N4-nitro-L-arginine (20), indicates that not only exercise hyperemia but also exercise capacity depends on the integrity of the NOS pathway.

That exercise hyperemia is dependent on the integrity of the NOS pathway has been both supported and refuted by earlier studies. Persson et al. (24) showed that hyperemia in response to electrically stimulated contractions was unaffected by infusion of NOS inhib-
is restricted by flow-limiting stenoses and coronary vasomotor dysfunction. L-Arginine likely restores the vasomotor function sufficiently to improve blood flow and delay the onset of ischemia. L-Arginine infusion has also been shown to improve walking distance in patients with intermittent claudication (4). Similar to patients with coronary artery disease, exercise capacity of patients with claudication is limited by both atherosclerotic lesions and EDNO-mediated vasomotor dysfunction.

We were surprised to observe the effect that supplemental L-arginine had on the aerobic capacity of healthy mice. The magnitude of the increase in exercise capacity achieved by L-arginine was equivalent to that produced by exercise training mice for 2 h daily, 6 days/wk for 4 wk (22). Exercise training has been shown by Sessa and colleagues (27) to enhance EDNO activity. Our finding leads us to speculate that prolonged intense exercise may lead to a state of NOS impairment that becomes limiting to oxygen uptake and aerobic capacity. The mechanism of the impairment could be one or more of those proposed for vascular diseases (i.e., production of an endogenous inhibitor of NOS, increased superoxide generation, or depletion of L-arginine in the region of NOS) (21). Regardless of the mechanism, this finding could have important implications for the athlete.

Critique of methods. Several limitations to this study are worth noting. It was our intention to use the D enantiomer of arginine, which is not a substrate for NOS inhibitor. In contrast, Dyke et al. (7) reported a diminution of forearm exercise hyperemia during prolonged handgripping exercises in humans with infusion of NOS inhibitor. Similarly, Hirai and colleagues (11) reported that exercise hyperemia in certain rat hindlimb muscles, as measured by delivery of radioactive microspheres, is dependent on EDNO release. Other studies suggest that EDNO is involved at low-intensity but not at high-intensity exercise (23) or is only partly responsible for exercise hyperemia because NOS inhibitors only partially reverse hyperemia (13).

That exercise capacity is dependent on the integrity of the NOS pathway is supported in the literature as well. For example, several investigators have shown that the endothelial dysfunction that occurs in heart failure can be reversed by measures that restore EDNO activity and, through this process, blood flow and exercise capacity are improved (9, 12, 14, 16, 26). Although these measures restore EDNO activity, they likely also act to improve cardiac function in heart failure, and this effect may also contribute to enhanced exercise capacity in these patients. In the present study, the hypercholesterolemic mice had evidence of impaired endothelial vasodilator dysfunction and EDNO activity but no evidence of impairment of cardiac function (as determined by organ chamber studies and cardiac histology, data not shown). Thus the effect of L-arginine was likely due to its ability to restore EDNO activity.

Our finding is consistent with the accumulating data indicating that supplemental L-arginine has a beneficial effect on exercise capacity in other conditions in which the NOS pathway is disturbed. Of particular note is the finding of increased maximum workload attained during treadmill testing before the onset of ST segment depression in patients with coronary artery disease (6). In this clinical condition, exercise capacity

Fig. 2. Bar graph of postexercise urinary nitrate concentration normalized to urinary creatinine concentration in wild-type (E+) and apolipoprotein E-deficient (E−) mice with N, L, and D supplementation conditions. Values are means ± SE. *P < 0.05 vs. N of same strain by ANOVA; §P < 0.05 vs. E+ by ANOVA.
served in the normocholesterolemic mice. In our study, the L-arginine-treated mice demonstrated an 8% increase in VO2 max with a similar increase in running distance. In contrast, the D-arginine-treated mice increased their VO2 max no more than 2% yet they increased their running distance by 30%. This observation might be explained by an excessive production of nitric oxide in the heavily L-arginine-supplemented mice. In addition to promoting exercise hyperemia, nitric oxide plays a role in cardiac and skeletal myocyte function. In these cells, a reduction in nitric oxide stimulates, whereas excess nitric oxide uncouples, mitochondrial respiration (5, 18, 28). Therefore, although vasodilation and delivery of oxygen may be enhanced with moderate increases in plasma L-arginine, excessive L-arginine availability may reduce the efficiency of oxygen utilization by myocytes. In our study, racemization may have been a rate-limiting step to excessive oxygen utilization by myocytes. To conclude, L-arginine enhances systemic nitric oxide production and increases aerobic exercise capacity in normal and hypercholesterolemic mice. This finding supports the role of EDNO in mediating exercise hyperemia and in determining aerobic capacity. Furthermore, in conditions whereby EDNO activity is reduced, there may be a benefit of L-arginine supplementation on exercise capacity.

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