Physical Activity Prevents Age-Related Impairment in Nitric Oxide Availability in Elderly Athletes

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Background—Aging is associated with increased cardiovascular risk and endothelial dysfunction. Since exercise can improve endothelium-dependent vasodilation, in the present study we tested whether long-term physical activity could prevent aging-related endothelial dysfunction.

Methods and Results—In 12 young and elderly (age 26.9±2.3 and 62.9±5.8 years, respectively) healthy sedentary subjects and 11 young and 14 elderly matched athletes (age 27.5±1.9 and 66.4±6.1 years, respectively), we studied (with strain-gauge plethysmography) forearm blood flow modifications induced by intrabrachial acetylcholine (0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL per minute), an endothelium-dependent vasodilator, at baseline, during infusion of N^G-monomethyl-L-arginine (L-NMMA) (100 μg/100 mL forearm tissue per minute), a nitric oxide–synthase inhibitor, vitamin C (8 mg/100 mL forearm tissue per minute), an antioxidant, and finally under simultaneous infusion of L-NMMA and vitamin C. The response to sodium nitroprusside (1, 2, and 4 μg/100 mL forearm tissue per minute) was also evaluated. In young athletes and sedentary subgroups, vasodilation to acetylcholine was inhibited by L-NMMA and was not changed by vitamin C. In elderly subjects, vasodilation to acetylcholine was blunted as compared with young subjects in both control subjects and athletes, whereas the response to sodium nitroprusside was similar. Moreover, in elderly athletes, vitamin C did not change the vasodilation to acetylcholine. In contrast, in elderly sedentary subjects, the response to acetylcholine was resistant to L-NMMA. In this subgroup, vitamin C increased the vasodilation to acetylcholine and restored the inhibiting effect of L-NMMA.

Conclusions—These results suggest that regular physical activity can at least in part prevent the age-induced endothelial dysfunction, probably the restoration of nitric oxide availability consequent to prevention of production of oxidative stress. **(*Circulation*. 2000;101:2896-2901.)**

Key Words: endothelium ■ nitric oxide ■ free radicals ■ antioxidants ■ exercise

Aging is a well-documented cardiovascular risk factor. One of the possible physiopathological mechanisms through which increasing age may lead to cardiovascular damage is the promotion of endothelial dysfunction. The endothelium plays a primary role in the modulation of vascular tone and structure[1] through production of the relaxing factor nitric oxide (NO), which acts by protecting the vessel wall from the development of atherosclerosis and thrombosis. A dysfunctioning endothelium, characterized by reduced NO availability induced by oxidative stress, can in the presence of most of the cardiovascular risk factors, including aging, be a promoter of atherosclerosis.[2,3] Moreover, endothelial dysfunction has been linked to the classic manifestations of established coronary artery disease.[2–4]

In humans, age-related impairment in endothelium-dependent vasodilation has been well documented in the forearm[5–7] and coronary[8,9] vascular bed. Moreover, at least in the forearm circulation of aged individuals, impaired endothelium-dependent vasodilation is associated with an alteration in the L-arginine–NO pathway.[7] Recent evidence indicates that physical exercise can improve endothelium-dependent vasodilation both in healthy humans[10] and in patients with endothelial dysfunction associated with chronic heart failure.[11] Thus, the aim of the present study was to evaluate whether regular physical activity could improve endothelium-dependent vasodilation by restoring NO availability and whether the mechanism responsible for this possible beneficial effect could be related to antioxidant activity.

Methods

Patients

The study population included 24 healthy subjects (mean age 53.8±16.1 years; blood pressure 121.4±6.3/78.2±3.2 mm Hg) and 25 matched normotensive athletes (mean age 54.4±17.4 years; blood pressure 123.5±6.7/77.6±3.0 mm Hg). Individuals smoking >5 cigarettes per day and/or consuming >60 g of ethanol (correspond-

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TABLE 1. Clinical Characteristics of Study Populations

	Sedentary Subjects		Athletes	
	Young (n=12)	Elderly (n=12)	Young (n=11)	Elderly (n=14)
Age, y	26.9±2.3	62.9±5.8	27.5±1.9	66.4±6.1
Sex, male/female	8/4	8/4	8/3	10/4
SBP, mm Hg	118.5±4.9	119.3±5.7	119.3±5.7	125.4±6.6
DBP, mm Hg	76.5±2.9	78.1±2.8	75.8±31	78.8±2.4
Heart rate, bpm	67.2±5.8	71.8±7.3	52.6±4.3*	54.7±5.3*
BMI, kg/m^2	22.6±3.7	24.1±4.4	23.8±4.8	23.8±4.7
Total cholesterol, mg/dL	174.3±15.9	189.3±23.8	165.4±13.9	181.5±16.7
HDL cholesterol, mg/dL	41.2±3.6	37.3±6.1	57.3±4.6*	59.4±5.1*
LDL cholesterol, mg/dL	101.3±10.6	110.2±13.8	71.8±12.9*	84.9±9.8*
Plasma glucose, mg/dL	79.4±7.1	73.4±11.1	83.9±8.3	86.4±9.1
$\dot{V}O_2$ max, mL · kg^{-1} · min^{-1}	40.2±2.1	38.2±2.2	68.2±2.9†	63.7±2.4†

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; and $\dot{V}O_2$ max, maximal O_2 consumption.

*$P<0.05$ or †$P<0.01$ vs sedentary group.

ing to half a liter of wine) per day were likewise excluded from the study.

Athletes (triathletes, long-distance runners, and cyclists) were selected on the basis of maximum oxygen consumption ($\dot{V}O_2$max) >60 mL · min^{-1} · kg^{-1}, whereas sedentary subjects performed no regular exercise and had a $\dot{V}O_2$max <45 mL · min^{-1} · kg^{-1}. $\dot{V}O_2$max was assessed during a graded exercise test on a cycle ergometer as previously described.[12] In each group, we enrolled young (<30 years of age) and elderly (>60 years of age) individuals characterized by similar age, sex, and body mass index (Table 1). The protocol was approved by the Ethics Committee of the University of Pisa, and all patients gave written consent to the study.

Experimental Procedure

Vascular reactivity was assessed by the perfused forearm technique. Briefly, the brachial artery was cannulated for drug infusion at systemically ineffective rates, intra-arterial blood pressure, and heart rate monitoring. Forearm blood flow (FBF) was measured in both forearms (experimental forearm and contralateral forearm) by strain-gauge venous plethysmography[13] during the last minute of each infusion period. Circulation to the hand was excluded 1 minute before FBF measurement by inflating a pediatric cuff around the wrist at suprasystolic blood pressure. Forearm volume was measured according to the water displacement method. Details concerning the method have already been published.[14]

Experimental Design

To assess endothelial function and evaluate whether oxygen free radicals can impair NO-mediated endothelium-dependent vasodilation, a dose-response curve to acetylcholine (cumulative increase of infusion rates: 0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL forearm tissue per minute for 5 minutes at each dose) was performed according to the following experimental design: during saline (0.2 mL/min), in the presence of intra-arterial N^G-monomethyl-L-arginine (L-NMMA) (100 μg/100 mL forearm tissue per minute), to block NO-synthase[15] in the presence of intra-arterial vitamin C (8 mg/100 mL forearm tissue per minute), an antioxidant,[16] and finally in the presence of simultaneous infusion of L-NMMA and vitamin C. In addition, endothelium-independent vasodilation was also assessed by a dose-response curve to intra-arterial sodium nitroprusside, a direct smooth muscle cell relaxant compound[17] (cumulative increase by 1, 2, and 4 μg/100 mL forearm tissue per minute for 5 minutes at each dose). Both L-NMMA and vitamin C were started 10 minutes before acetylcholine and continued throughout. This infusion time was chosen on the basis of previous evidence demonstrating that it is sufficient to obtain a stable vascular effect.[14,18] FBF was measured before starting and during the last (9th) minute of L-NMMA or vitamin C infusion. When acetylcholine was coinfused, FBF was measured during the last (5th) minute of each acetylcholine infusion rate. A 30-minute washout was allowed between each dose-response curve, whereas a 60-minute period was allowed when L-NMMA was infused.

Data Analysis

Since arterial pressure did not significantly change during the study, all data were analyzed in terms of FBF, as absolute values, and percent increase or decrease above baseline. Clinical characteristics of the study subjects were compared by the paired and unpaired Student's *t* test. Dose-response curves to acetylcholine and sodium nitroprusside were analyzed by ANOVA for repeated measures, and Scheffé test was applied for multiple comparison testing. Results are expressed as mean±SD. Computations for the statistical method described were performed with the use of the SAS System.

Results

Apart from age, the 4 study subgroups were comparable for sex distribution, blood pressure, body mass index, and plasma total cholesterol and glucose values (Table 1). However, trained individuals showed a decreased resting heart rate, increased and decreased plasma HDL and LDL cholesterol values, respectively, and, in accordance with the inclusion criteria, an increased $\dot{V}O_2$max as compared with sedentary subjects (Table 1).

Acetylcholine-induced increase in FBF was found to be significantly ($P<0.000001$) blunted in elderly sedentary individuals as compared with young control subjects (Figure 1). Elderly athletes also showed a decreased ($P<0.001$) response to acetylcholine as compared with young athletes (Figure 1). Thus, whereas in young sedentary and athletic individuals the vasodilating effect of acetylcholine was similar (Figure 2), in the elderly subgroups athletes showed a greater ($P<0.001$) response to the muscarinic agonist as compared with elderly sedentary control subjects (Figure 2) (Table 2).

The vasodilating effect of sodium nitroprusside was also similar in both young subgroups (Figure 2). It is worth noting

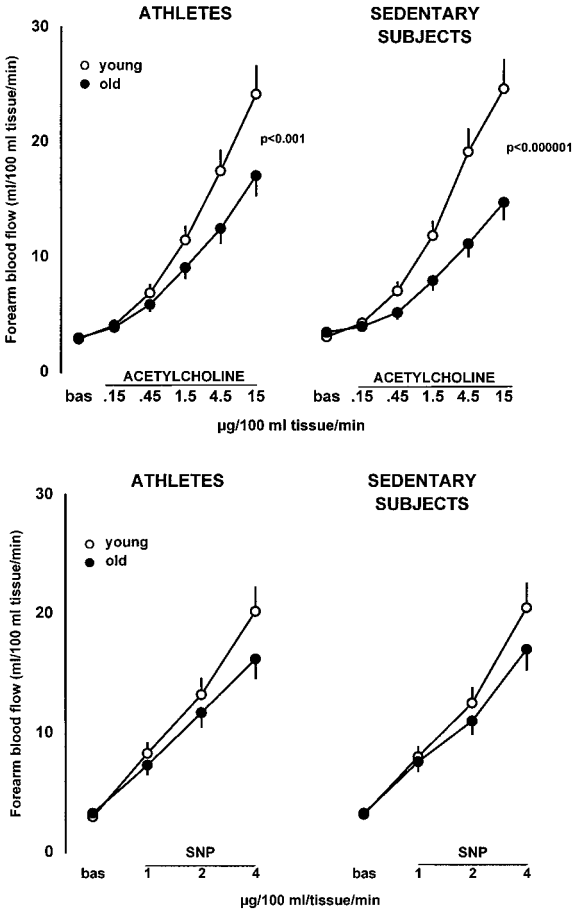

Figure 1. Line graphs show forearm vasodilation induced by intrabrachial administration of acetylcholine (0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL tissue per minute) (top) and sodium nitroprusside (SNP, 1, 2, and 4 μg/100 mL tissue per minute) (bottom) in young (<30 years, ○) and elderly (>60 years, ●) athletes and sedentary subjects. Statistical difference was calculated by ANOVA.

that in the elderly individuals the response to sodium nitroprusside was slightly and nonsignificantly reduced as compared with the young population (Figure 1), with no difference between athletes and sedentary subjects (Table 2).

In young athletes and sedentary subjects, L-NMMA infusion caused a similar significant ($P<0.01$) decrease in basal FBF and blunted the vasodilating effect of acetylcholine ($P<0.0000001$ versus acetylcholine alone) (Figure 3) (Table 2). The degree of L-NMMA–induced inhibition of vasodilation to acetylcholine was similar in the 2 subgroups. In elderly subjects, although L-NMMA–induced decrease in basal FBF was significantly ($P<0.05$) reduced as compared with young individuals, the vasoconstrictor effect of the NO-synthase inhibitor was found to be significantly ($P<0.05$) greater in athletes as compared with sedentary control subjects. Moreover, L-NMMA significantly ($P<0.00001$) blunted the vasodilation to acetylcholine in elderly athletes but was ineffective in elderly sedentary control subjects (Figure 3) (Table 2).

In the overall study population, vitamin C did not change basal FBF or the vasoconstrictor effect induced by L-NMMA. Moreover, in both young and elderly athletes and in young sedentary control subjects, vitamin C changed neither the vasodilation to acetylcholine nor the degree of L-NMMA–induced inhibition of the dose-response curve to the muscarinic agonist (Figure 4) (Table 2). In contrast, in elderly sedentary control subjects, vitamin C increased ($P<0.01$) the vasodilation induced by acetylcholine and restored the inhibiting ability of L-NMMA on the response to the agonist (Figure 4) (Table 2).

In both normotensive subjects and essential hypertensive patients, contralateral FBF did not significantly change throughout the study (data not shown).

Discussion

In control elderly individuals, vasodilation to acetylcholine but not to sodium nitroprusside, an endothelium-dependent and endothelium-independent agonist, respectively, is blunted as compared with young subjects, thus confirming previous evidence demonstrating the presence of endothelial dysfunction associated with advancing age in humans.[5–9] Analysis of the effect of physical activity showed that in young, trained individuals, vasodilation to acetylcholine was

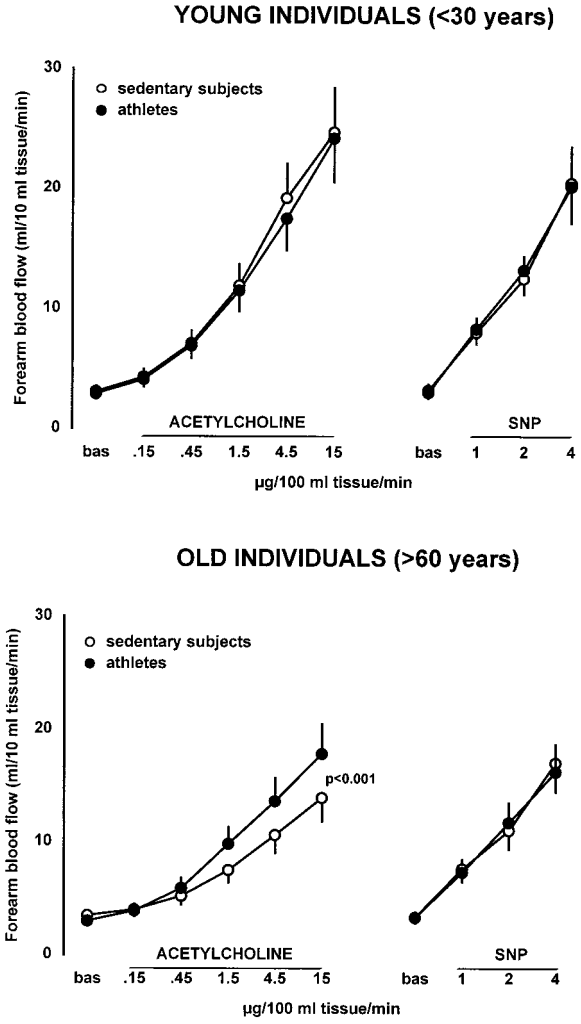

Figure 2. Line graphs show forearm vasodilation induced by intrabrachial administration of acetylcholine (0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL tissue per minute) (left) and sodium nitroprusside (SNP, 1, 2, and 4 μg/100 mL tissue per minute) (right) in young (<30 years, top) and elderly (>60 years, bottom) athletes (●) and sedentary subjects (○). Statistical difference was calculated by ANOVA.

TABLE 2. Forearm Blood Flow Changes Induced by Sodium Nitroprusside and Acetylcholine

	Sedentary Subjects		Athletes	
	Young	Elderly	Young	Elderly
Baseline	3.2±0.5	3.3±0.6	3.0±0.5	3.3±0.6
Sodium nitroprusside, 4 μg/100 mL tissue per minute	20.5±3.9	16.2±3.2	20.2±3.7	17.0±3.0
%Change	540±36	390±36	573±36	415±36
Baseline	3.1±0.6	3.5±0.5	2.9±0.6	3.0±0.4
Acetylcholine, 15 μg/100 mL tissue per minute	24.7±4.4	14.8±5.6	24.2±5.5	17.1±4.2
%Change	713±27	341±36†	753±37	457±28*
Baseline	3.1±0.6	3.7±0.5	3.1±0.6	3.1±0.4
L-NMMA	1.6±0.4	2.5±0.4	1.5±0.5	1.8±0.3
%Change	−49±8‡	−32±11†‡	−51±9‡	−42±7*‡
Acetylcholine	5.8±1.7	9.5±3.6	4.6±0.9	6.1±1.1
%Change	262±22§	283±26	208±33§	238±20§
Baseline	3.1±0.5	3.2±0.7	3.0±0.6	3.1±0.4
Vitamin C	3.2±0.4	3.2±0.7	3.0±0.5	3.1±0.4
Acetylcholine	23.8±4.2	18.9±4.5	24.0±3.6	18.0±3.9
%Change	667±31	498±33§	701±33	480±27
Baseline	3.2±0.6	3.2±0.7	3.0±0.6	3.1±0.4
Vitamin C	3.2±0.7	3.2±0.7	3.0±0.5	3.1±0.4
Vitamin C+L-NMMA	1.7±0.4	2.4±0.7	1.5±0.5	1.8±0.3
%Change	−47±7‡	−25±9*‡	−50±8*‡	−42±7*‡
Acetylcholine	6.0±2.0	10.1±2.5	4.7±1.1	6.0±1.0
%Change	253±25‖	318±33‖	213±29‖	233±22‖

Response to acetylcholine was tested at baseline and in the presence of L-NMMA (100 μg/100 mL tissue/per minute), vitamin C (8 mg/100 mL tissue/per minute), and simultaneous infusion of L-NMMA and vitamin C. Results are expressed as absolute values (mL/100 mL forearm tissue/per minute) and as % modifications above baseline.

*$P \leq 0.05$ vs young individuals, †$P \leq 0.001$ vs athletes, ‡$P \leq 0.05$ vs baseline, §$P \leq 0.01$ vs acetylcholine alone, ‖$P \leq 0.01$ vs acetylcholine+vitamin C.

not different from that observed in matched sedentary control subjects, whereas in elderly trained subjects the response to acetylcholine, although reduced as compared with young athletes, was significantly greater than in matched elderly sedentary subjects. In contrast, no difference in the vasodilating effect of sodium nitroprusside was found between trained and control individuals. L-NMMA blunted the response to acetylcholine in young trained and sedentary individuals, confirming that in healthy conditions, at least a substantial part of the vasodilating effect of acetylcholine is mediated by the availability of NO.[14,15] In this respect, it is noteworthy that in elderly sedentary individuals, L-NMMA produced a lesser degree of forearm vasoconstriction, an indirect marker of tonic NO release, as compared with elderly athletes and young sedentary control subjects and athletes, and failed to blunt the reduced response to acetylcholine, demonstrating the presence of impaired basal and receptor activated NO availability. However, in elderly athletes, L-NMMA was still able to inhibit vasodilation to acetylcholine, demonstrating that physical activity can prevent the reduction in NO availability that is characteristic of impaired endothelium-dependent vasodilation in elderly individuals. Finally, in young athletes and sedentary subjects, vitamin C, which blocks oxidative stress by a scavenger activity,[16] did not change the response to acetylcholine or the inhibiting effect exerted by L-NMMA on the endothelial agonist, indicating that oxidative stress plays no major role in affecting endothelial responses in young individuals, independent of physical training. On the other hand, in elderly trained individuals, vitamin C was still ineffective in modifying the vasodilation to acetylcholine-induced or L-NMMA–induced inhibition, whereas in elderly sedentary subjects the antioxidant significantly increased the response to the muscarinic agonist and, perhaps more importantly, restored the inhibiting capability exerted by L-NMMA. Taken together, these results demonstrate that sedentary elderly subjects are characterized by the presence of age-related endothelial dysfunction caused by oxidative stress–induced reduction in NO availability. In these clinical conditions, long-term physical training appears to reverse this alteration by preventing oxidative stress and thereby preserving NO availability.

The present results also seem to suggest that in young age groups, endothelial function, at least in the forearm microcirculation, is preserved and cannot be affected by potentially

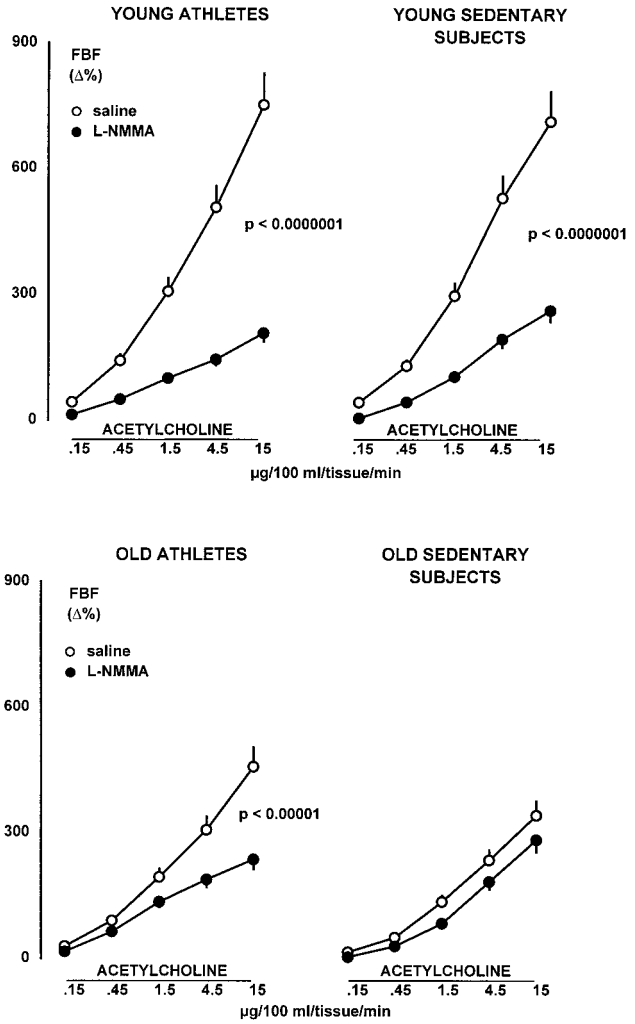

Figure 3. Line graphs show forearm vasodilation induced by intrabrachial administration of acetylcholine (0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL tissue per minute) during saline (0.2 mL/min, ○) or L-NMMA (●) administration in young (<30 years) and elderly (>60 years) athletes and sedentary subjects. Results are represented as percentage of FBF changes compared with baseline. Statistical difference was calculated by ANOVA.

beneficial interventions such as physical training. This finding is at variance with experimental evidence demonstrating that the expression and activity of NO-synthase is increased by physical training and associated with increased NO-dependent vasodilation.[19,20] There are several possible explanations for this discrepancy. First, in young subjects, NO-synthase may work at a maximum rate that cannot be further increased. In agreement with this hypothesis, previous evidence demonstrates that in subjects <30 years of age, that is, a study population comparable with the present one, forearm endothelium-dependent vasodilation cannot be improved by clinical conditions, such as the presence of endogenous estrogen,[21] or pharmacological intervention, such as L-arginine supplementation,[7] whereas these appear to be effective in individuals >30 years of age. A second very plausible explanation could be related to the fact that we evaluated endothelial function in a vascular district (forearm) different from that specifically trained (legs) in our study population, composed essentially of cyclists and runners. Previous evidence indicates that local physical activity can selectively improve vascular reactivity in the specifically trained vascular bed.[22,23] If this is the case, in our experimental conditions, the systemic beneficial effect of exercise may not be sufficiently strong to induce a positive effect in a nonspecifically trained vascular district. In contrast, in elderly subjects, the beneficial effect of physical training may have been detectable because of the presence of a more pronounced endothelial dysfunction. A final third possibility could be related to insufficient sensitivity of the experimental method for measurement of a small beneficial effect.

Previous experimental and human evidence indicates that physical training is associated with increased endothelium-dependent vasodilation.[20,24,25] Moreover, long-term exercise can improve the endothelial function even in patients with chronic heart failure, a clinical condition characterized by impaired endothelium-dependent vasodilation.[11] At partial

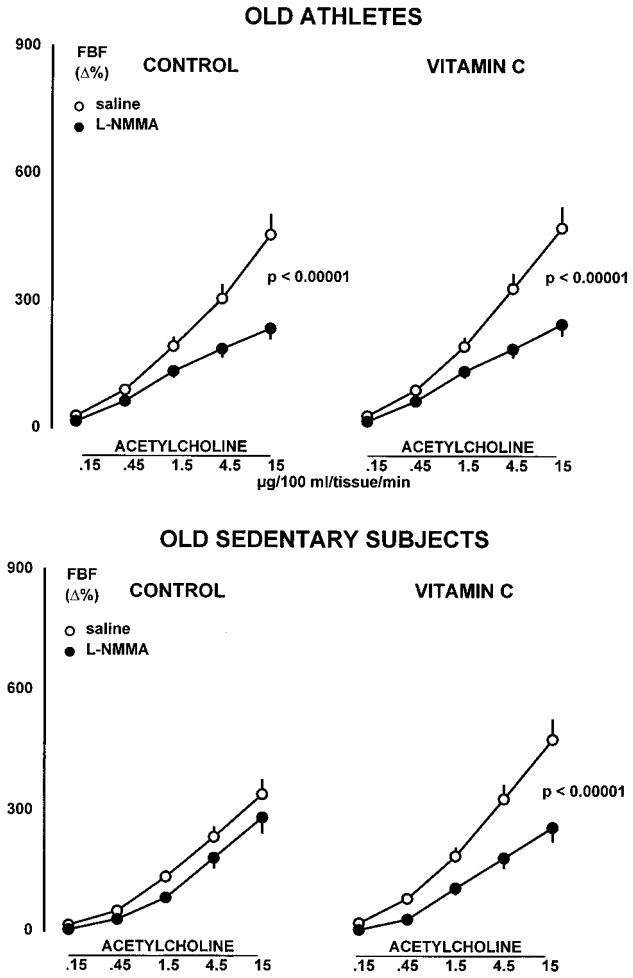

Figure 4. Line graphs show forearm vasodilation induced by intrabrachial administration of acetylcholine (0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL tissue per minute) during saline (0.2 mL/min, ○) or L-NMMA (●) administration, in absence (control) and presence of vitamin C (8 mg/100 mL per minute) in elderly athletes (>60 years) (top) and elderly sedentary subjects (>60 years) (bottom). Results are represented as percentage of FBF changes compared with baseline. Statistical difference was calculated by ANOVA.

variance with the present results demonstrating no effect of long-term physical training on endothelial function in young individuals, Clarkson et al[10] demonstrated that 10-week exercise training improved endothelium-dependent, flow-mediated dilation in the brachial artery of young (mean age 20 years) sedentary subjects. Possible explanations for these conflicting results could be related to the different vascular district explored (microcirculation versus macrocirculation) or the different stimulus used to activate endothelial function (increase in shear stress versus receptor stimulation).

As regards the mechanism through which physical exercise can partially correct age-related endothelial dysfunction, the present results seem to indicate that long-term training prevents oxidative stress production and the consequent reduction in NO availability. This possibility is in agreement with a large body of evidence indicating that the state of physical training can per se modulate organic antioxidant defenses.[26] Another possible mechanism could be related to the well documented[27,28] improvement in lipid profile exerted by physical training, which is confirmed in our long-term–trained study population. Moreover, long-term exercise decreases LDL susceptibility to oxidation.[29] However, it should be considered that if a better lipid profile were responsible for a preserved endothelial function in elderly athletes, such a mechanism would be operative in all patients with impaired NO availability.

Finally, as a possible study limitation, it must be noted that in a cross-sectional study such as the present one, the preserved endothelial function in the senior athletic population may not be related to the physical training but could instead be the expression of genetic selection.

The beneficial effect of exercise on endothelium-dependent vasodilation and NO availability can have important clinical implications. It is well documented that a preserved endothelial function can protect the vessel wall from the development of atherosclerosis and thrombosis,[1,2] whereas a dysfunctional endothelium can negatively act as a promoter of atherosclerotic vascular damage.[2–4] Therapeutic intervention that improves endothelial function could therefore have a beneficial impact on cardiovascular disease. In this respect, it has recently been demonstrated that dynamic exercise (regularly walking >1.5 miles per day) reduces cardiovascular risk in the elderly.[30] It is tempting to speculate that part of the beneficial effect of this training physical program could be related to an improvement in endothelial function.

In conclusion, the present study demonstrates that regular physical training protects the vascular endothelium from aging-related alterations. The beneficial effect of exercise is related to preservation of NO availability by a mechanism probably linked to the prevention of oxidative stress and the consequent NO breakdown. This beneficial effect could be important in accounting for the positive impact of regular exercise on cardiovascular risk in the elderly population.

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