

# Physical Activity, Plasma Antioxidant Capacity, and Endothelium-Dependent Vasodilation in Young and Older Men

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**Background:** Sedentary aging is associated with oxidative stress and endothelial dysfunction. The aim of this study was to evaluate the relationship between long-term physical activity, plasma antioxidant status, and conduit artery endothelial function in young and older healthy men.

**Methods:** In young ( $n = 16$ ) and older athletes ( $n = 16$ ) and in matched healthy sedentary subjects, endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent response to glyceryl trinitrate (GTN), 400  $\mu\text{g}$ , were measured in the brachial artery from high-resolution ultrasonography. Plasma malondialdehyde (MDA) and antioxidant capacity as total oxyradical scavenging capacity (TOSC) were also evaluated.

**Results:** We found that FMD was lower ( $\leq 0.01$ ) in sedentary older subjects ( $2.3\% \pm 1.0\%$ ) as compared with older athletes ( $5.3\% \pm 3.2\%$ ) and both sedentary ( $5.4\% \pm 2.0\%$ ) and athletically trained ( $6.1\% \pm 3.2\%$ ) young sub-

jects. Sedentary older subjects showed higher ( $P \leq .05$ ) MDA levels and lower ( $P < .0001$ ) plasma antioxidant capacity as compared with the other subgroups, whereas in older athletes MDA levels and antioxidant capacity were similar to those observed in the young subgroups. In the whole group, FMD, but not GTN, was negatively related to age ( $r = -0.31$ ,  $P < .05$ ) and directly related ( $P \leq .01$ ) to  $\text{VO}_2\text{max}$  ( $r = 0.49$ ) and TOSC against peroxy ( $r = 0.69$ ) and hydroxyl radicals ( $r = 0.53$ ). In the multivariate analysis, TOSC against peroxy radicals resulted as the most significant predictor of FMD ( $R^2 = 0.60$ ;  $P = .003$ ).

**Conclusions:** These results suggest that regular physical activity is associated with preserved antioxidant defenses and endothelial function in older individuals. Am J Hypertens 2005;18:510–516 © 2005 American Journal of Hypertension, Ltd.

**Key Words:** Endothelium, vasodilation, arteries, physical activity, free radicals.

**E**ndothelium plays an important role in the local regulation of vascular tone and structure, mainly by nitric oxide (NO) synthesis and action.<sup>1</sup> Endothelial dysfunction has been associated with several risk factors for atherosclerosis including dyslipidemia, diabetes, essential hypertension, and smoking.<sup>2–5</sup>

Aging is an independent risk factor for the development of atherosclerosis and is associated with a progressive decline in endothelium-dependent vasodilation in both resistance<sup>6–9</sup> and conduit vessels.<sup>10,11</sup> This alteration is related to a worsening of oxidative status that pertains to both increased oxygen free radicals production<sup>9,11</sup> and a gradual loss of antioxidant capacity,<sup>12</sup> leading together to impaired NO availability.<sup>9</sup>

Physical training has beneficial effects on multiple cardiovascular risk factors such as dyslipidemia, hypertension, diabetes, and cardiovascular events.<sup>13</sup> The effect of exercise on clinical outcome could also be partially related to a direct and independent positive effect of physical training on endothelial dysfunction in the conduit arteries<sup>14</sup> or in the peripheral microcirculation.<sup>15–18</sup> Thus, regular physical activity is associated with increased endothelium-dependent vasodilation<sup>19</sup> and NO availability.<sup>15–18</sup> This beneficial effect is likely to be related to oxidative stress, as short-term vitamin C administration can improve endothelium-dependent vasodilation in the peripheral microcirculation<sup>17</sup> and conduit arteries<sup>11</sup> in older sedentary individuals while being ineffective in athletes of the same age. In this regard, available evidence supports

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**Table 1.** Clinical characteristics of the study population (mean  $\pm$  SD)

	Young Sedentary Subjects (n = 16)	Older Sedentary Subjects (n = 16)	Young Athletes (n = 16)	Older Athletes (n = 16)
Age (y)	34.1 $\pm$ 7.5	63.7 $\pm$ 4.3	33.4 $\pm$ 6.7	63.6 $\pm$ 6.1
BMI (kg/m <sup>2</sup> )	23.1 $\pm$ 1.2	24.2 $\pm$ 1.3	23.4 $\pm$ 0.7	23.9 $\pm$ 1.0
Heart rate (beats/min)	61.5 $\pm$ 4.6	65.3 $\pm$ 9.2	49.6 $\pm$ 4.3*	53.9 $\pm$ 5.2*
SBP (mm Hg)	119.3 $\pm$ 4.5	126.2 $\pm$ 5.4	119.4 $\pm$ 4.3	126.3 $\pm$ 4.2
DBP (mm Hg)	75.3 $\pm$ 7.6	78.7 $\pm$ 5.8	75.5 $\pm$ 3.1	78.2 $\pm$ 5.2
Glucose (mg/dL)	91.8 $\pm$ 7.7	93.1 $\pm$ 9.0	89.6 $\pm$ 8.4	92.0 $\pm$ 0.9.1
Total cholesterol (mg/dL)	183.9 $\pm$ 10.5	187.7 $\pm$ 12.0	182.1 $\pm$ 9.0	185.9 $\pm$ 11.3
HDL cholesterol (mg/dL)	60.5 $\pm$ 8.7	43.4 $\pm$ 8.7†	63.3 $\pm$ 11.1	58.9 $\pm$ 11.3
LDL cholesterol (mg/dL)	99.9 $\pm$ 12.2	109.4 $\pm$ 10.2†	101.7 $\pm$ 12.1	101.4 $\pm$ 12.8
VO <sub>2</sub> max (mL/kg min)	37.9 $\pm$ 2.9	28.0 $\pm$ 5.9†	66.8 $\pm$ 2.9‡	54.7 $\pm$ 3.7
Brachial artery diameter (mm)	5.02 $\pm$ 0.53	5.07 $\pm$ 0.71	5.43 $\pm$ 0.57	5.44 $\pm$ 0.74
Reactive hyperemia (%)	534 $\pm$ 211	513 $\pm$ 222	567 $\pm$ 190	565 $\pm$ 227

BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure; VO<sub>2</sub>max = maximal O<sub>2</sub> uptake.

\*  $P < .0001$  v sedentary subgroups; †  $P < .0001$  v other subgroups; ‡  $P < .0001$  v older subgroups.

the possibility that the beneficial effect of physical exercise on oxidative stress might be related to increased antioxidant defenses.<sup>20</sup>

However, no single study has simultaneously addressed the relationship between aging, oxidative stress, antioxidant defenses, and physical activity on endothelial function in humans. The aim of the present study was therefore to evaluate in young and older athletes and healthy sedentary subjects the relationship between flow-mediated endothelium-dependent dilation (FMD) of the brachial artery, a plasma marker of oxidative stress and plasma antioxidant activity.

## Methods

### Study Population

The study population included 16 young male athletes (mean age 33.4  $\pm$  6.7 years) and 16 older male athletes (mean age 63.6  $\pm$  6.1 years) and age- and sex-matched healthy sedentary subjects (Table 1). Subjects were included in the study in the absence of cardiovascular disease or risk factors. Clinical history, physical examination, basal and stress electrocardiography, blood chemistry, and biochemistry evaluations were performed. No subjects were smokers or used medications or vitamin supplementation.

Athletes were considered for the study when maximal oxygen uptake (VO<sub>2</sub>max), assessed by a graded exercise test (cycle ergometer), was  $>50$  mL/min/kg, whereas sedentary subjects were included when VO<sub>2</sub>max was  $<45$  mL/min/kg.<sup>21</sup> Young and older endurance-trained subjects had engaged in exercise for 11  $\pm$  2 and 37  $\pm$  5 years, respectively. They performed vigorous endurance exercise ( $>5$  times/week) and were active in national and international road-running races; all subjects were recruited from running clubs throughout the surrounding regions and from the National Veterans Sport Club of Pisa.

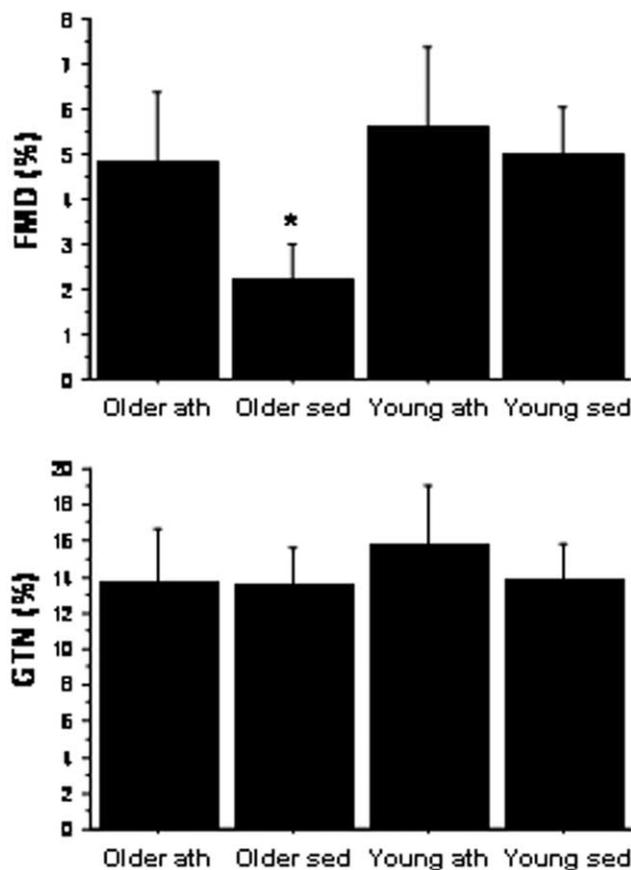
Sedentary subjects were recruited through various forms of advertisement. The protocol was approved by the Ethics Committee of the University of Pisa, and all patients gave written consent to the study.

### Experimental Procedure

Vascular ultrasonographic scans were performed in the morning, with subjects supine, in a quiet air-conditioned room (22° to 24°C). A B-mode scan of the right brachial artery was obtained in a longitudinal section between 5 cm and 10 cm above the elbow using a 7.0-MHz linear-array transducer and a standard AU5 Armonic system (ESAOTE Biomedica, Florence, Italy), as previously described.<sup>4</sup> Briefly, the transducer was held at the same point throughout the scan by a stereotactic clamp. End-diastolic frames were acquired every 1 sec on a personal computer using a commercial software program (miro VIDEO DC30/plus, Pinnacle Systems GmbH, Braunschweig, Germany). Arterial flow velocity was obtained by pulsed Doppler signal at 70 degrees to the vessel with the range gate (1.5 mm) in the center of the artery. A cuff was placed around the forearm just below the elbow.

### Experimental Protocol

Endothelium-dependent response was assessed as dilation of the brachial artery induced by increased flow (flow-mediated dilation [FMD]).<sup>2,22</sup> After 1 min of baseline acquisition, the cuff was inflated for 5 min and then deflated to induce reactive hyperemia (RH). Endothelium-independent dilation was obtained by sublingual administration of glyceryl trinitrate (GTN), 400  $\mu$ g. Brachial artery diameter was measured on acquired frames by a computerized edge detection system.<sup>23</sup> The FMD and response to GTN were calculated as the maximal percent increase in diameter above baseline after RH and GTN administration, respectively.



**FIG. 1.** Flow-mediated dilation (FMD) and glyceryl trinitrate (GTN) induced dilation, expressed as maximal percentage change of brachial artery diameter, in the young and older subgroups of athletes and sedentary subjects (mean  $\pm$  SD). \* $P < .001$  v other subgroups.

Blood flow volume was calculated by multiplying Doppler flow velocity (corrected for the angle), heart rate, and vessel cross-sectional area ( $\pi r^2$ ) at baseline and within 15 sec after cuff release to calculate RH (as percent increase in blood flow).

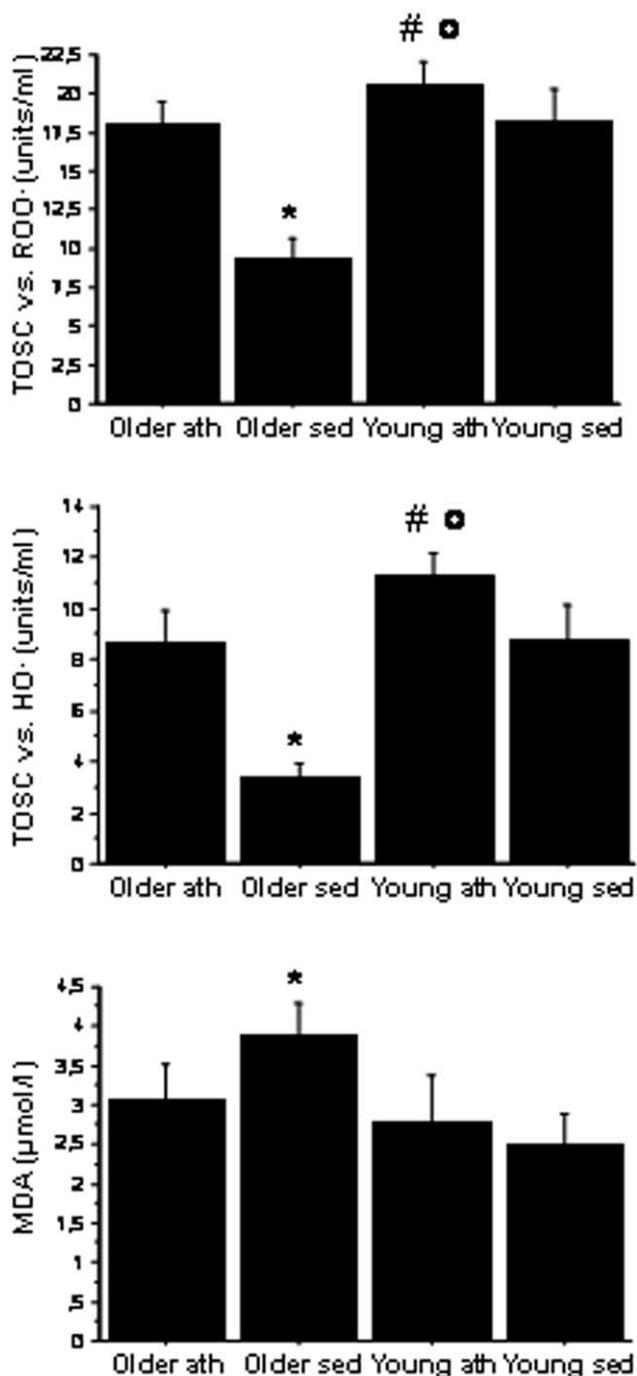
Blood samples were collected following a 12-h overnight fast. A total oxyradical scavenging capacity (TOSC) assay was used for measuring the total antioxidant capacity of human plasma against peroxy radicals ( $\text{ROO}\cdot$ ) and hydroxyl radicals ( $\text{HO}\cdot$ ) by gas-chromatographic analysis (6890 series, Hewlett-Packard, Andover, MA), as previously described.<sup>24,25</sup> Results were expressed in TOSC units per milliliter of plasma. In our laboratory, the variability of TOSC assay was  $<5\%$ .<sup>26</sup>

Oxidative stress was evaluated by measuring lipid peroxidation of polyunsaturated fatty acids in terms of thiobarbituric acid-reactive substances and converted into malondialdehyde (MDA).<sup>27</sup> The amount of lipid peroxidation was reported as micromolar MDA equivalents.

### Statistical Analysis

Data are expressed as means  $\pm$  SD. Analysis of variance was used to assess mean differences between

groups. Differences were considered significant at values of  $P < .05$ . Interactions between variables were calculated by correlation and multiple regression analyses. All statistical procedures were performed using the StatView program (Abacus Concepts, Inc., SAS Institute, Cary, NC).



**FIG. 2.** Plasma total oxyradical scavenging capacity (TOSC) against peroxy radicals ( $\text{ROO}\cdot$ ) and hydroxyl radicals ( $\text{HO}\cdot$ ), and plasma malondialdehyde (MDA) in the young and older subgroups of athletes and sedentary subjects (mean  $\pm$  SD). \* $P < .001$  v others subgroups, # $P < .01$  v young sedentary subjects, ° $P < .01$  v older athletes.

## Results

Clinical characteristics of the study population are shown in Table 1. Sedentary subjects and athletes were matched for arterial blood pressure and body mass index. According to the inclusion criteria,  $\text{VO}_2\text{max}$  was higher in trained subjects than in sedentary subgroups. Athletes also showed a lower resting heart rate than nonathletes. The sedentary older subgroup showed significantly lower HDL and higher LDL cholesterol than did the other subgroups.

Older sedentary subjects showed lower FMD than older athletes and both young subgroups (Fig. 1), whereas no differences were shown in the response to GTN (Fig. 1). Both FMD and response to GTN were similar in young and older athletes. The RH and brachial artery diameter were similar in the four study groups (Table 1).

Older sedentary subjects exhibited lower TOSC values  $\nu$   $\text{ROO}\cdot$  and  $\nu$   $\text{HO}\cdot$  ( $-48\%$  and  $-61\%$ , respectively) and higher MDA plasma levels ( $+26\%$ ) as compared with the young sedentary subgroup and both trained subgroups (Fig. 2). Older athletes showed significantly lower TOSC  $\nu$   $\text{ROO}\cdot$  and  $\nu$   $\text{HO}\cdot$  but not significantly higher MDA levels as compared with young athletes, whereas this difference was not observed between older athletes and young sedentary subjects (Fig. 2).

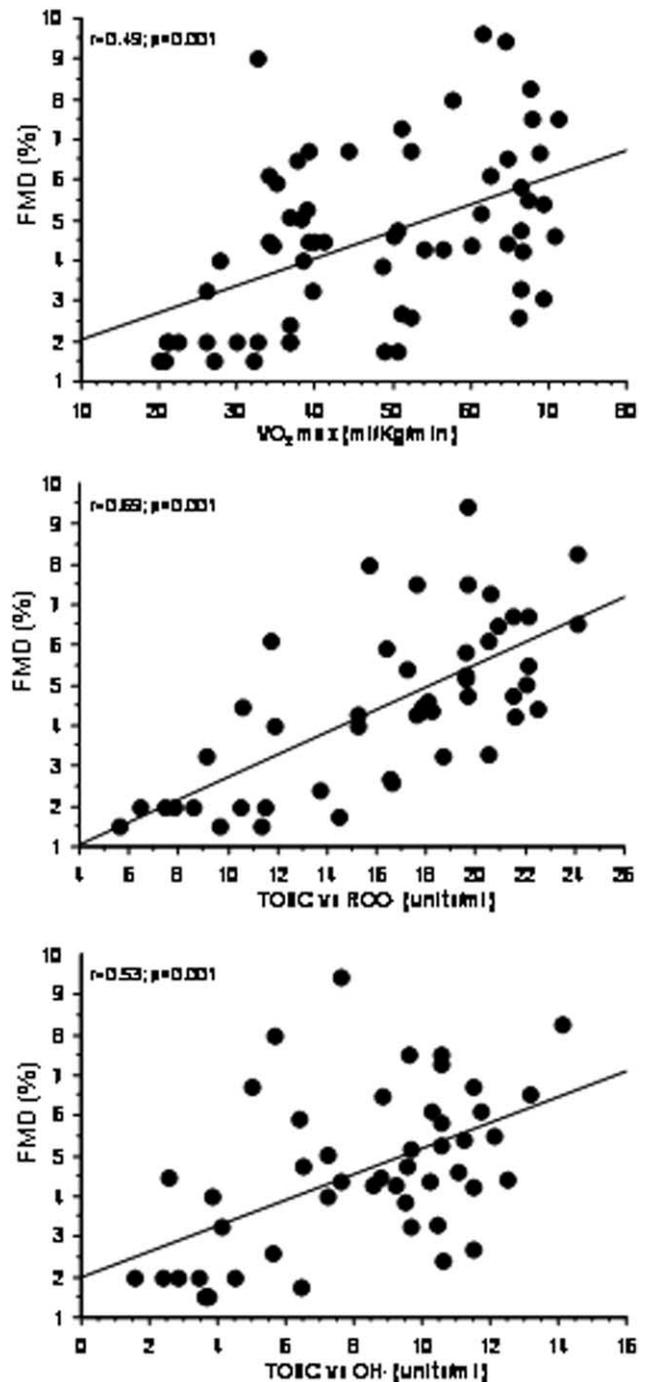
### Univariate and Multivariate Analysis: Whole Population

In the whole population, FMD, but not GTN response, was significantly related to  $\text{VO}_2\text{max}$ , TOSC versus  $\text{ROO}\cdot$  and versus  $\text{HO}\cdot$  and was inversely related to age ( $r = -0.31$ ;  $P < .05$ ) (Fig. 3). In addition, FMD was positively related to HDL cholesterol ( $r = 0.37$ ,  $P < .01$ ) and inversely related to LDL cholesterol ( $r = -0.27$ ;  $P < .05$ ).  $\text{VO}_2\text{max}$  was significantly related to TOSC versus  $\text{ROO}\cdot$  ( $r = 0.73$ ;  $P < .001$ ) and versus  $\text{HO}\cdot$  ( $r = 0.72$ ;  $P < .001$ ) and was inversely related to MDA levels ( $r = -0.30$ ;  $P < .05$ ). Furthermore, age was directly related to MDA ( $r = 0.44$ ;  $P < .01$ ) and inversely related to TOSC versus  $\text{ROO}\cdot$  ( $r = -0.53$ ;  $P < .001$ ), and versus  $\text{HO}\cdot$  ( $r = -0.57$ ;  $P < .001$ ) and  $\text{VO}_2\text{max}$  ( $r = -0.32$ ;  $P < .05$ ).

In a multivariate analysis, both age and  $\text{VO}_2\text{max}$  were shown to be significant predictors of FMD ( $R^2 = 0.25$ ;  $P = .0001$ ). However,  $\text{VO}_2\text{max}$  accounted for a larger amount of variance in FMD ( $R^2 = 0.13$ ,  $P = .004$ ) than did age ( $R^2 = 0.07$ ;  $P = .04$ ). Including all significant variables, only TOSC value  $\nu$   $\text{ROO}\cdot$  proved to be a significant predictor of FMD ( $R^2 = 0.60$ ;  $P = .003$ ).

### Subgroup Analysis

In the sedentary subjects, FMD was inversely related to age (Fig. 4). It was also related to  $\text{VO}_2\text{max}$  ( $r = 0.51$ ;  $P < .001$ ), TOSC versus  $\text{ROO}\cdot$  ( $r = 0.70$ ;  $P < .001$ ) and versus



**FIG. 3.** Correlation analysis between flow mediated dilation (FMD), expressed as maximal percentage change of brachial artery diameter, maximal oxygen uptake ( $\text{VO}_2\text{max}$ , **top**), and plasma total oxylradical scavenging capacity (TOSC) against peroxyl radicals ( $\text{ROO}\cdot$ , **middle**) and hydroxyl radicals ( $\text{HO}\cdot$ , **bottom**).

$\text{HO}\cdot$  ( $r = 0.47$ ;  $P < .01$ ) and HDL ( $r = 0.48$ ;  $P < .01$ ). In the multivariate analysis, TOSC value versus  $\text{ROO}\cdot$  was shown to be the only significant predictor of FMD ( $R^2 = 0.64$ ;  $P = .02$ ).

In the athletes, FMD was not related to age (Fig. 4), whereas it was related to TOSC versus  $\text{ROO}\cdot$  ( $r = 0.37$ ;  $P = .05$ ) and  $\text{VO}_2\text{max}$  ( $r = 0.34$ ;  $P = .05$ ).

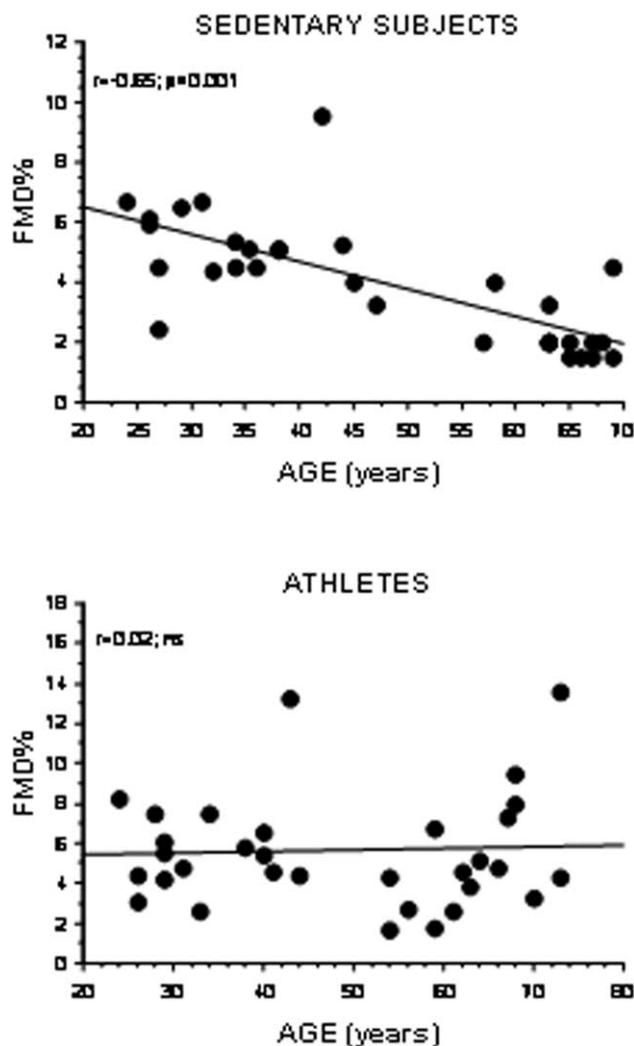


FIG. 4. Correlation analysis between flow mediated dilation (FMD, maximal percent increase) and age in sedentary subjects (top) and athletes (bottom).

## Discussion

The present study indicates that advancing age is characterized by an impairment of endothelium-dependent FMD of conduit arteries with a parallel attenuation of plasma antioxidant capacity and increased production of MDA, a marker of oxidative stress. In contrast, in older subjects who regularly perform aerobic endurance training, FMD is preserved. This beneficial effect seems to be related to a higher plasma antioxidant capacity, which can counteract the oxidative stress associated with aging.

Older sedentary subjects showed a reduced endothelium-dependent FMD as compared with that in young subjects, whereas endothelium-independent vasodilation was similar. These findings confirm previous research showing the presence of impaired endothelium-dependent vasodilation in the brachial artery of older sedentary subjects.<sup>10,11</sup> This age-related impairment of endothelium-dependent vasodilation could be caused either by a decreased production of NO, because of changes in expression or to activity of

eNOS,<sup>28,29</sup> or an increased degradation of NO by reactive oxygen species.<sup>30–32</sup>

In the population of sedentary subjects, the older subgroup exhibited a higher level of MDA, a plasma marker of oxidative stress, as compared with the young subgroup. Moreover, endothelium-dependent FMD was inversely related to MDA, thus supporting the possibility that the age-related increase of oxidative stress could be a promoter of endothelial dysfunction. These results are in line with previous studies showing that ascorbate can acutely restore endothelium-dependent vasodilation in the brachial artery<sup>11</sup> and in the forearm microcirculation<sup>17</sup> in older sedentary subjects but not in older athletes.

Moreover, aging is associated with a gradual loss of antioxidant capacity,<sup>12</sup> which normally provides cellular protection against reactive oxygen species. The evaluation of the TOSC assay, a highly reproducible method for quantitative measurement of the capacity of a molecule or a tissue to neutralize various classes of reactive oxygen species,<sup>25</sup> showed that plasma antioxidant defense capacity against both peroxy and hydroxyl radicals is reduced in sedentary aging. In addition, plasma antioxidant defense capacity against peroxy radicals was only a negative predictor of FMD, suggesting that the loss of antioxidant capacity associated with aging might represent the main determinant of age-related impairment in endothelium-dependent vasodilation.

In line with previous studies,<sup>11,19</sup> older athletes showed a higher FMD than sedentary matched subjects. Moreover, in older athletes, FMD was not significantly different with respect to both young subgroups, confirming that endothelial dysfunction is slowed in older subjects by endurance exercise training for many years. Thus, the demonstrated beneficial effects of regular dynamic physical exercise are in line with previous evidence in both conduit arteries<sup>14</sup> and peripheral microcirculation.<sup>15–18</sup> However, the interesting and novel finding of this study is that preserved FMD in older athletes coincides with a higher antioxidant capacity and lower oxidative stress as compared with that in older sedentary subjects.

Although it has been suggested that short-term bouts of exercise can cause oxidative stress by increasing LDL susceptibility to oxidation or vascular superoxide production,<sup>33</sup> long-term exercise exerts beneficial effects on oxidative stress, ameliorating lipid profile, a phenomenon related to physical activity,<sup>34</sup> or increasing antioxidant defenses.<sup>20</sup> In our population, maximal oxygen uptake was positively related to HDL and inversely related to LDL cholesterol. We found that HDL cholesterol was strongly related to plasma antioxidant capacity, supporting the hypothesis that regular endurance training could reduce susceptibility to oxidative stress by modifying lipid profile. However, because plasma antioxidant capacity against peroxy radicals was the only significant predictor of FMD, it is conceivable that other mechanisms might account for this effect, including up-regulation of superoxide dismutase<sup>35</sup> or other antioxidant defenses.<sup>36,37</sup>

It is interesting to observe that young athletes had higher TOSC plasma levels than their sedentary peers, whereas FMD was not significantly different (6.2% v 5.4%, respectively) in the two subgroups. This discrepancy might be tentatively explained by the possibility that although antioxidant defenses are susceptible to increase, FMD may reach a maximal physiologic value that is difficult to increase. Another explanation could be related to the evidence that individuals undergoing exercise training have high levels of antioxidant enzymes and nonenzymatic antioxidants, causing a greater resistance to exercise-induced oxidative stress.<sup>20</sup> Presumably, these adaptations result from cumulative effects of repeated exercise bouts on the gene expression of antioxidant enzymes,<sup>20</sup> despite the observed low oxidative levels.

In conclusion, the preserved antioxidant capacity and endothelium-dependent vasodilation observed in older athletes suggest that long-term physical activity can counteract the age-related endothelial dysfunction that characterizes sedentary aging, probably by maintaining plasma antioxidant defenses and thereby preventing oxidative stress.

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