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# Physical activity alters antioxidant status in exercising elderly subjects

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**Abstract**

Nutritional adequacy and physical activity are two aspects of a health-promoting lifestyle. Not much is known about antioxidant nutrient requirements for exercising elderly (EE) subjects. The question of whether exercise training alters the status of antioxidant vitamins as well as trace elements in elderly subjects and fails to balance the age-related increase in oxidative stress is addressed in this study. There were 18 EE (68.1±3.1 years), 7 sedentary elderly (SE; 70.4±5.0 years), 17 exercising young (EY; 31.2±7.1 years) and 8 sedentary young (SY; 27.1±5.8 years) subjects who completed 7-day food and activity records. Each subject's blood was sampled on Day 8. A similar selenium (Se) status but a higher erythrocyte glutathione peroxidase (GSH-Px) activity were found in EE subjects as compared with EY and SE subjects. Blood oxidized glutathione was higher and plasma total thiol was lower in EE subjects as compared with EY subjects. Mean vitamin C (167 vs. 106 mg/day), vitamin E (11.7 vs. 8.3 mg/day) and β-carotene (4 vs. 2.4 mg/day) intakes were higher in EE subjects as compared with EY subjects. However, EE subjects exhibited the lowest plasma carotenoid concentrations, especially in β-carotene, which was not related to intakes. Despite high intakes of antioxidant micronutrients, no adaptive mechanism able to counteract the increased oxidative stress in aging was found in EE subjects. Results on GSH-Px activity illustrate that the nature of the regulation of this biomarker of Se status is different in response to training and aging. These data also strongly suggest specific antioxidant requirements for athletes with advancing age, with a special attention to carotenoids.


*Keywords:* Aging; Carotenoids; Dietary intakes; Exercise; Oxidative stress; Selenium

## 1. Introduction

In young athletes, aerobic exercise training is reported to reinforce the endogenous antioxidant defense system. In fact, the mild oxidative stress induced by repeated bouts of aerobic exercise stimulates the expression and increases the de novo protein synthesis of some antioxidant enzymes [1]. It is still unknown whether these adaptive mechanisms differ in elderly and young subjects, and it can be supposed that the endogenous antioxidant system in exercising elderly (EE) subjects is not relevant for the same adaptive mechanisms as compared with that in exercising young subjects [2]. In healthy elderly subjects, it is not clear whether habitual physical activity favorably affects antioxidant potential. For some authors, exercise training prevents lipid peroxidation [3,4], whereas some others did not find evidence of any effect of exercise training on lipoperoxidative markers after a 12-week program [5]. Moreover, training-induced adaptation on the antioxidant system is also influenced by nutritional factors in overloaded subjects [6,7]. In well-trained competitive athletes, antioxidant status is preserved by adequate dietary antioxidant intakes [8]. In EE subjects, changes in absorptive and metabolic capacity might influence their food choice and could lead to deficiencies in antioxidant vitamins and trace elements [9,10]. To date, recommended dietary allowances for EE subjects cannot be provided because there is still a substantial lack of data regarding exercise training effects in elderly subjects [11]. The age-related adaptation failure in response to exercise training could be aggravated in cases of inadequate antioxidant intakes. The question of whether exercise training alters the status of antioxidant vitamins as well as trace elements in elderly subjects and fails to balance the age-related increase in oxidative stress is addressed in this study.

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## 2. Materials and methods

### 2.1. Subjects

There were 18 healthy EE (68.1±3.1 years), 7 sedentary elderly (SE; 70.4±5.0 years), 17 exercising young (EY; 31.2±7.1 years) and 8 sedentary young (SY; 27.1±5.8 years) subjects who participated in the study. The characteristics of the subjects are shown in Table 1. All subjects were males. Only athletes involved in a regular aerobic physical activity, who have a minimum of three training sessions per week and with each training session lasting >1 h were selected. As a control group, sedentary subjects with no regular physical activity for a minimum of 3 years were recruited. Excluded from this study were smokers, regular alcohol users, subjects with a hypoenergetic or hyperenergetic diet, subjects with a body mass index (BMI) >35, subjects with a medical illness or with a family history of coronary heart disease and subjects receiving prescription medication or using antioxidants or multivitamins and trace element supplements for at least 6 months prior to the study.

Participants were all asked to maintain their normal behavior during the 7-day follow-up. Subjects were recruited from a pool of volunteers. Elderly subjects were all recruited from the same sports association. The subjects were predominantly of middle socioeconomic class, with middle or higher levels of education and were living in the region of Provence-Alpes-Côte d'Azur (France). There was an attempt to balance the study population according to habitual physical activity. The survey was developed in agreement with the 1975 Declaration of Helsinki, which has been reviewed in 1989. The survey received the approval of the Protective Committee of People in the Biomedical Research (No. 02.002). Subjects were informed of the nature of the progress of the experimentation before soliciting their formal consent.

### 2.2. Diet and activity records

A 7-day food record and a 7-day activity record were completed by each subject on a notebook. At the start, a standardized individual information session was organized to give subjects instructions on how to record their daily food intake. Food quantities were estimated, specifying the number of units and a code corresponding to the size of the portion, using a reference portion guideline book [12]. Parallel with the food record, subjects kept a 7-day activity record. Activities were divided into the following groups: (1) personal activities; (2) home and leisure activities; (3) locomotion; (4) professional, school or voluntary activities; and (5) physical training. A scale of intensity was included. Subjects reported each morning in the same conditions their body mass to control energy balance stability. On Day 8, 24-h diet and activity recalls were carried out face-to-face by trained interviewers—all of whom were experts in nutrition—and lasted approximately 30 min. The average nutrient diet content was calculated using Regal Micro software (Version 1.2, Max Feinberg, France). For carotenoids (lutein, zeaxanthin, lycopene, β-cryptoxanthin and α-carotene), intakes were estimated with the use of the European carotenoid database of O'Neill et al. [13]. Intakes of selenium (Se), zinc (Zn) and copper (Cu) were estimated with the use of the French database of Lamand et al. [14]. Estimated intakes were referred to the French Recommended Dietary Allowance for Physically Active People (FRDAa), which was recently established by the French Food Agency of Sanitary Security [15]. FRDAs have been adjusted in a linear way by adding 12 mg, 100 mg, 1000 μg, 30 μg and 1 mg for vitamin E, vitamin C, β-carotene, Se and Zn, respectively, per additional 4180 kJ of energy expenditure >9200 kJ for males. The Compendium of Physical Activities [16] was used to provide the energy cost of physical activity expressed as metabolic equivalents (METs). Physical activity, expressed as total daily energy expenditure, was calculated by multiplying the amount of time spent in each activity and the corresponding METs. Any activity that could not be found in the database was carefully evaluated to determine the most appropriate corresponding activity.

Physical training in the EE and EY groups permitted the subjects to reach significantly higher daily energy expenditures as compared with the SE and SY groups. The EE and EY subjects had similar physical activities and energy expenditures (Table 1).

Table 1
Characteristics of the subjects[a]

| | SY ($n$=8) | SE ($n$=7) | EY ($n$=17) | EE ($n$=18) |
|---|---|---|---|---|
| Age (years) | 27.1±5.8 | 70.4±5.0*** | 31.2±7.1** | 68.1±3.1*,*** |
| Weight (kg) | 67.3±11.3 | 69.6±8.2 | 66.4±5.3** | 77.4±7.0*,*** |
| BMI (kg/m$^2$) | 21.7±2.3 | 24.3±1.9 | 21.8±1.5** | 25.6±2.3*,*** |
| Total cholesterol (g/L) | 1.89±0.21 | 2.24±0.21 | 1.74±0.27** | 2.16±0.31* |
| HDL cholesterol (g/L) | 0.53±0.04 | 0.56±0.09 | 0.56±0.08 | 0.54±0.10 |
| LDL cholesterol (g/L) | 1.19±0.19 | 1.48±0.20 | 1.05±0.22** | 1.44±0.28* |
| Triglycerides (g/L) | 0.89±0.32 | 0.98±0.39 | 0.72±0.17 | 0.92±0.25 |
| Total DEE (MJ/day) | 9.76±1.30 | 10.18±0.76 | 13.22±0.98**,*** | 13.19±1.15**,*** |
| Training load (h/week) | 0 | 0 | 11.5±3.2 | 10.7±4.3 |

[a] Values are expressed as mean±S.D. DEE, daily energy expenditure.
* $P$<.05, different from EY.
** $P$<.05, different from SE.
*** $P$<.05, different from SY.

Table 2
Total daily EI, macronutrient, fiber, fruit and vegetable intakes and alcohol consumption in all subjects[a]

| | SY ($n$=8) | SE ($n$=7) | EY ($n$=17) | EE ($n$=18) |
|---|---|---|---|---|
| EI (MJ/day) | 9.90±2.31 | 10.26±1.84 | 12.64±2.24** | 11.49±2.44** |
| Carbohydrates | | | | |
| % EI | 42.9±6.0 | 43.3±6.1 | 49.0±5.6*** | 41.5±7.4* |
| g/day | 253±66 | 264±60 | 371±86**,*** | 281±61* |
| Proteins | | | | |
| % EI | 16.0±2.4 | 15.1±2.4 | 15.6±2.4 | 16.5±2.2 |
| g/kg of BW/day | 1.42±0.38 | 1.42±0.42 | 1.77±0.37*** | 1.46±0.33* |
| Animal (% total proteins) | 72±4 | 65±33 | 67±7 | 62±29 |
| Vegetal (% total proteins) | 28±4 | 35±33 | 33±7 | 38±29 |
| Lipids | | | | |
| % EI | 35.7±7.1 | 34.4±6.3 | 33.2±4.4 | 34.7±6.2 |
| g/day | 96±33 | 94±25 | 111±24 | 108±37 |
| Saturated fatty acid (g/day) | 38.2±15.5 | 36.9±10.2 | 43.9±10.8 | 44.4±17.5 |
| Monounsaturated fatty acid (g/day) | 35.5±10.8 | 30.5±7.5 | 39.1±10.3 | 39.3±13.3 |
| Polyunsaturated fatty acid (g/day) | 11.4±4.0 | 15.0±7.8 | 13.4±3.2 | 12.8±4.1 |
| Cholesterol (mg/day) | 422±100 | 417±176 | 405±133 | 495±169 |
| Fiber (g/day) | 15.8±3.6 | 18.2±4.0 | 21.2±9.1** | 25.1±7.5**,*** |
| Fruits (g/day) | 150±58 | 158±20 | 214±116**,*** | 286±111**,*** |
| Vegetables (g/day) | 129±81 | 144±87 | 200±145 | 255±165 |

[a] Values are expressed as mean±S.D. EI, energy intake; BW, body weight.
* $P$<.05, different from EY.
** $P$<.05, different from SE.
*** $P$<.05, different from SY.

### 2.3. Blood sampling

On Day 8, blood samples were collected by puncture from the antecubital vein in vacutainer tubes (Becton Dickinson, Le pont de Claix, France) after an overnight fast between 8 and 10 AM and at least 12 h after the last exercise session. Blood samples were collected in heparinized tubes protected from light and in Zn-free tubes.

Blood was centrifuged immediately at room temperature for 10 min at 3000×$g$. The supernatant was then transferred to cryotubes for measurement of plasma ascorbic acid, α-tocopherol and carotenoid concentrations as well as to specific Zn-free tubes for measurement of plasma Se and Zn concentrations. Cryotubes were kept at −80°C and metal-free tubes were kept at −20°C until measurement within 6 months. For glutathione (GSH) measurement, immediately after venipuncture, 400 μl of whole blood was transferred into a tube containing 3600 μl of metaphosphoric acid (60 ml/L water). The content was mixed and centrifuged for 10 min at 4°C. Acidic protein-free supernatant fractions were stored at −80°C until analysis.

### 2.4. Biologic assays

#### 2.4.1. Plasma antioxidant vitamins and trace elements

Ascorbic acid concentrations were measured by fluorometry using an automated method in plasma after stabilization and extraction with a 5% metaphosphoric acid solution according to Speek et al. [17]. α-Tocopherol and carotenoid concentrations were determined by high-performance liquid chromatography as described by Arnaud et al. [18].

Table 3
Antioxidant micronutrient intakes in all subjects[a]

| | SY ($n$=8) | SE ($n$=7) | EY ($n$=17) | EE ($n$=18) |
|---|---|---|---|---|
| Vitamin C (mg/day) | 87±58 | 100±33 | 109±62 | 167±73*,**,*** |
| Vitamin E (mg/day) | 8.0±3.2 | 9.0±3.5 | 8.3±2.7 | 11.7±4.1*,*** |
| β-Carotene (mg/day) | 1.55±1.07 | 3.12±2.06 | 2.42±1.35 | 4.00±2.38* |
| Lycopene (mg/day) | 2.59±1.41 | 3.65±3.18 | 3.87±3.45 | 3.91±2.92 |
| Lutein (mg/day) | 0.49±0.30 | 1.09±0.88 | 1.32±0.56 | 1.96±1.38*** |
| α-Carotene (mg/day) | 1.17±0.16 | 1.12±0.71 | 1.24±1.57 | 1.18±0.58 |
| Total carotenoids (mg/day) | 4.56±2.37 | 8.98±5.97*** | 8.86±3.78 | 11.64±3.65*** |
| Zn (mg/day) | 9.5±1.9 | 8.7±1.3 | 13.4±3.8 | 13.5±4.7** |
| Cu (mg/day) | 1.08±0.14 | 1.36±0.44 | 1.57±0.75 | 1.68±0.59 |
| Se (μg/day) | 58.3±12.1 | 67.9±14.0 | 75.1±26.6 | 70.3±22.7 |

[a] Values are expressed as mean±S.D.
* $P$<.05, different from EY.
** $P$<.05, different from SE.
*** $P$<.05, different from SY.

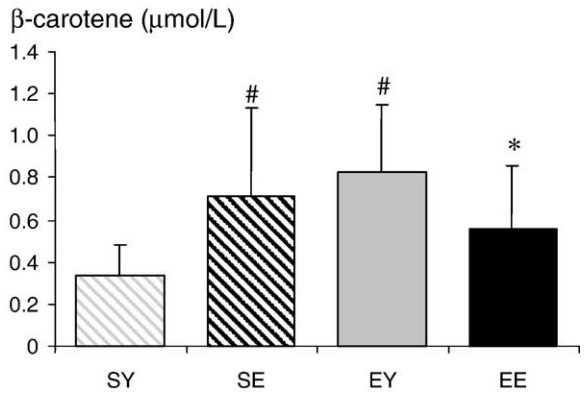


Fig. 1. Plasma β-carotene concentrations in SY (▨), SE (▨), EY (■) and EE (■) subjects. *$P$<.05, different from EY. #$P$<.05, different from SY.

Plasma Se concentrations were determined using a Perkin Elmer 5100 (Norwalk, CT, USA) fitted with an HGA 600 furnace, an electron discharge lamp and a Zeeman background correction [19]. Plasma Zn was determined by flame atomic absorption spectrometry as previously described [20]. Inter-assay coefficients of variation were 2.9% and 9% for Se and Zn, respectively.

### 2.4.2. Erythrocyte antioxidant metalloenzymes

Erythrocyte glutathione peroxidase (GSH-Px) activity was evaluated using terbutyl hydroperoxide (Sigma, Via Coger, Paris, France) as a substrate instead of hydrogen peroxide [21]. Results are expressed as micromoles of NADPH (Boehringer-Mannheim, Germany) oxidized per minute per gram of hemoglobin. Intra-assay and inter-assay coefficients of variation were 1.46% and 1.76%, respectively.

Erythrocyte Cu–Zn superoxide (SOD) dismutase activity was measured after hemoglobin precipitation by monitoring the auto-oxidation of pyrogallol [22]. Intra-assay and inter-assay coefficients of variation were 2.5% and 1.4%, respectively.

Erythrocyte glutathione reductase (GR) activity was determined without adding FAD in the presence of an excess of $NADPH_2$ and with oxidized glutathione (GSSG) as a substrate [23].

### 2.4.3. Protein oxidation marker

Plasma total thiol group (-SH group) concentrations were assayed in 100 μl of plasma using 5,5′-dithiobis(2-nitrobenzoic acid) for deriving the -SH groups [24]. Within-run and between-run coefficients of variation were 1.58% and 3.5%, respectively.

### 2.4.4. Oxidative stress marker

Blood GSH concentrations were determined using enzymatic cycling of GSH by means of NADPH and GR coupled with 5,5′-dithiobis(2-nitrobenzoic acid). We estimated blood GSSG concentrations according to the method of Akerboom and Sies [25], which was slightly modified by Emonet et al. [26]. Intra-assay coefficients of variation were 2.8% and 6% and inter-assay coefficients of variation were 3.5% and 9% for total GSH and GSSG measurements, respectively.

Plasma HDL cholesterol, total cholesterol and triglyceride concentrations were determined by enzymatic color tests on Olympus analyzers. Within-run precision values of the method were 0.62%, 0.91% and 1.07% for HDL, total cholesterol and triglycerides, respectively.

### 2.5. Statistical analysis

All values are expressed as mean±S.D. One-way ANOVA tests were performed for comparisons between groups (SY, EY, EE and SE). When significant changes were observed in ANOVA tests, Fisher's PLSD post hoc test was applied to locate the source of significant differences. Owing to missing values in the EY group, the nonparametric Mann–Whitney test was performed for comparison between the EY and EE groups for blood GSH and GSSG concentrations. Bivariate associations between continuous variables were also assessed by linear regression analysis.

Analyses were performed with StatView Abacus Concept Version 5. The statistical significance level was set at $P$<.05.

Table 4
Antioxidant micronutrient plasma levels in all subjects[a]

| | SY ($n$=8) | SE ($n$=7) | EY ($n$=17) | EE ($n$=18) |
|---|---|---|---|---|
| α-Tocopherol (μmol/L) | 23.1±2.4 | 27.8±5.3 | 27.1±4.0 | 29.0±7.0** |
| Ascorbic acid (μmol/L) | 45.8±19.5 | 56.0±14.2 | 59.2±9.7 | 57.6±11.0 |
| Lycopene (μmol/L) | 80.69±0.26 | 0.43±0.17 | 0.74±0.52 | 0.41±0.16* |
| Lutein (μmol/L) | 0.214±0.068 | 0.269±0.088 | 0.391±0.137**·*** | 0.260±0.082* |
| α-Carotene (μmol/L) | 0.063±0.035 | 0.224±0.171** | 0.242±0.120** | 0.167±0.072*·** |
| Total carotenoids[b] | 1.31±0.42 | 1.63±0.54 | 2.16±0.63**·*** | 1.37±0.46* |
| Se (μmol/L) | 0.97±0.21 | 1.07±0.14 | 0.99±0.21 | 1.01±0.11 |
| Zn (μmol/L) | 11.9±1.1 | 12.9±1.2 | 12.9±1.5 | 12.1±1.3 |

[a] Values are expressed as mean±S.D.
[b] Sum of carotenoids quantified (β-carotene, lycopene, lutein and α-carotene).
* $P$<.05, different from EY.
** $P$<.05, different from SY.
*** $P$<.05, different from SE.

## 3. Results

### 3.1. Dietary intakes

The percentage of daily energy intake derived from carbohydrates was lower in EE subjects as compared with EY subjects (Table 2). EE subjects had lower carbohydrate intakes and lower protein intakes with respect to their body weight (Table 2).

In comparison with sedentary subjects, whatever their age, exercising subjects had higher daily total energy, fiber and fruit intakes (Table 2). EY subjects had significantly higher carbohydrate intakes in comparison with SY subjects, whereas no significant difference was found between the two groups of elderly subjects (SE and EE; Table 2).

Vitamins C and E and β-carotene intakes were higher in EE subjects as compared with EY subjects (Table 3). These two groups did not differ significantly in daily lutein, lycopene, α-carotene, Se, Zn and Cu intakes.

In comparison with SE subjects, EE subjects had significantly higher vitamin C and Zn intakes (Table 3). No significant difference in vitamin E, carotenoid, Se and Cu intakes was shown between those two groups. It is noteworthy that 61% of EE subjects reached the 2:3 of the French Recommended Dietary Allowances for physically active subjects (FRDAa) for vitamin C, 23% for vitamin E, 84% for β-carotene, 60% for Se, 87% for Zn and 40% for Cu. In EY subjects, 40% reached the 2:3 of the FRDA for vitamin C, 0% for vitamin E and 47% for β-carotene.

### 3.2. Biologic parameters

Total and LDL cholesterols were higher in both elderly groups (SE and EE) as compared with the EY group (Table 1). No difference was shown for HDL cholesterol between groups.

Plasma β-carotene, α-carotene, lycopene, lutein and total carotenoid concentrations were significantly lower in EE subjects in comparison with EY subjects (Fig. 1; Table 4). EY subjects had significantly higher plasma lutein, β-carotene, α-carotene and total carotenoid concentrations in comparison with SY subjects. However, no difference was found between EE and SE subjects in plasma carotenoid concentrations (Table 4). It is noteworthy that 22% of EE subjects had marginal β-carotene concentrations (<30 μmol/L). Plasma α-tocopherol, ascorbic acid, Zn and Se concentrations were not significantly different between groups (Table 4). All subjects, whatever their age and training status, had normal plasma α-tocopherol and ascorbic acid concentrations. A total of 12% of the elderly subjects and 12% of the younger subjects had plasma Zn concentrations <10.7 μmol/L. Plasma Se concentration was normal in the elderly groups, and 8% of young subjects had concentrations <0.76 μmol/L. Cu status appeared adequate as suggested by the lack of decline in Cu–Zn SOD activity.

With regard to enzymatic endogenous antioxidant status, erythrocyte Cu–Zn SOD activity did not differ between groups (Table 5). Erythrocyte GSH-Px activity was significantly higher in the EE group as compared with the other groups (Fig. 2). As erythrocyte GSH-Px activity is known to depend on Se status, in a second time, plasma Se concentration was introduced as a covariable in the ANOVA test. We evidenced a trend of interaction effect between groups and Se status on erythrocyte GSH-Px

Table 5
Enzymatic and nonenzymatic endogenous antioxidant status and oxidative stress markers in all subjects[a]

| | SY ($n$=8) | SE ($n$=7) | EY ($n$=17) | EE ($n$=18) |
|---|---|---|---|---|
| Cu–Zn SOD (U/mg Hb) | 1.44±0.04 | 1.32±0.04 | 1.35±0.14 | 1.36±0.09 |
| GR (U/g Hb) | 3.06±0.35 | 3.39±0.62 | 3.67±0.83 | 3.34±0.43 |
| GSH[b] (μmol/L) | 1042.3±137.7 | 901.0±127.6 | 871.0±164.8 | 952.7±184.6 |
| Total GSH[b] (μmol/L) | 1100±137 | 960±129 | 909±157 | 1010±195 |
| GSSG[b] (μmol/L) | 28.8±18.4 | 29.5±7.8 | 17.6±3.5[§] | 33.9±25.1* |
| GSH/GSSG[b] | 47.4±26.8 | 32.1±9.2 | 51.9±17.7 | 37.9±17.7 |

[a] Values are expressed as mean±S.D.
[b] Missing values for young subjects.
* $P$<.05, different from EY.
[§] $P$<.05, different from SE.

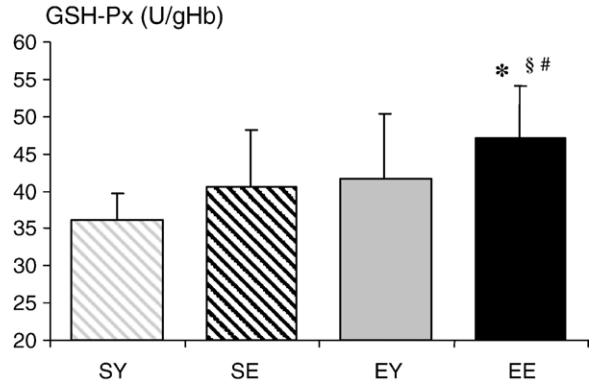


Fig. 2. Erythrocyte GSH-Px activities in SY (▨), SE (▧), EY (■) and EE (■) subjects. *$P$<.05, different from EY. [#]$P$<.05, different from SY. [§]$P$<.05, different from SE.

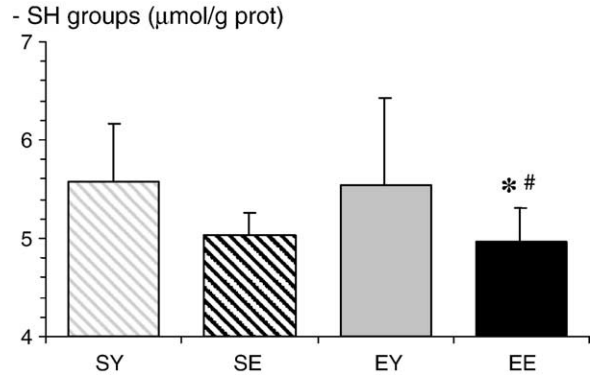


Fig. 3. Plasma thiol (-SH) group total concentrations in SY (▨), SE (▧), EY (■) and EE (■) subjects. *$P$<.05, different from EY. [#]$P$<.05, different from SY.

activity ($P$=.18). Erythrocyte GSH-Px activity was positively correlated to Se status in the SE group ($y$=37.1$x$+2.6; $n$=7; $r$=.82; $P$=.04). Erythrocyte GR activity and blood GSH concentrations did not differ significantly between groups (Table 5).

### 3.3. Oxidative stress and protein oxidation markers

EE subjects had lower plasma -SH group concentrations as compared with EY subjects (Fig. 3). Moreover, EE and SE subjects exhibited significant higher values of blood GSSG concentrations as compared with EY subjects (Table 5). EE subjects also tended to have a lower GSH/GSSG ratio ($P$=.15; Table 5). None of these parameters differed significantly between EE and SE subjects.

It is noteworthy that plasma Zn concentrations in EE subjects tended to be positively correlated to -SH group concentrations ($y$=3.52+0.12$x$; $n$=18; $r$=.43; $P$=.086).

## 4. Discussion

Nutritional adequacy and physical activity are two aspects of a health-promoting lifestyle. Not much is known about specific antioxidant nutrient requirements for EE subjects and about how they are modulated by physical activity. The age-related adaptation failure in response to exercise training could be aggravated in cases of inadequate antioxidant intakes.

Of the endogenous antioxidants measured, GSH-Px activity exhibited the highest variation in relation to age and training status. EE subjects had a higher erythrocyte GSH-Px activity as compared with their SE and EY counterparts. This confirms the age-associated [27,28] and aerobic training-associated [29–31] effects observed on erythrocyte GSH-Px activity. Interestingly, our data demonstrate that the adaptive increase in erythrocyte GSH-Px activity in response to exercise training is not altered with advancing age. Se constitutes the active center of GSH-Px and is incorporated in the form of selenocysteine during GSH-Px translation [32]. Se intakes and concentrations were not different between groups. Mean daily Se intakes in the elderly subjects in our study (70 μg) were higher than intakes (23 μg) quantified in French hospitalized elderly patients [33]. None of elderly subjects exhibited marginal plasma Se concentrations (<0.76 μmol/L), but plasma Se concentrations in most subjects were lower than the postulated concentration (1.15 μmol/L) to be required to maximize erythrocyte GSH-Px activity [34]. As previously shown [35], the dose–response association between plasma Se concentration and erythrocyte GSH-Px activity was not evidenced in athletes. However, this association was shown in SE subjects. Thus, training and aging effects on erythrocyte GSH-Px activity may not be relevant for the same mechanism. A higher steady-state concentration of cellular hydrogen peroxide ($H_2O_2$) with aging might consequently lead to increased induction of GSH-Px gene expression [36]. Sedentary and long-term trained old mice exhibited a similar increase in GSH-Px gene expression [37]. Posttranscriptional mechanisms can be proposed to mostly determine the higher GSH-Px activity observed with training status. The preservation of the active form of the GSH-Px enzyme as a result of the increased nitric oxide (NO) availability with physical training might be a mechanism explaining the higher erythrocyte GSH-Px activity observed in EE subjects. It has been shown that NO directly inactivates GSH-Px, resulting in an increase in intracellular peroxides, which in turn are responsible for cellular damage [36,38]. It is likely that in our study, EY and EE subjects differed according to their training status (e.g., type intensity of exercise). However, there was an attempt to balance the two exercising groups (young and old) according to the subjects' physical activity levels. It is known that physical training, by preventing oxidative stress, preserves NO availability [39]. Even if more experimental studies are needed to further elucidate the molecular basis of exercise training, this effect could be significant in accounting for the well-established positive impact of regular exercise on cardiovascular risk in the elderly population [40].

The lack of concomitant increase of erythrocyte GR and Cu–Zn SOD activities, together with the high blood GSSG concentrations, might be interpreted as an insufficient antioxidant defense in erythrocyte under high exercise training stress in EE subjects to cope with enhanced free radical production. Blood GSSG concentrations were higher in EE subjects in comparison with their EY and SE counterparts, without significant difference in total blood GSH. In aging, the oxidized form of GSH is enhanced [41]. However, our results in athletes differed from those of Kretzschmar et al. [42], who reported that aerobic exercise training partly compensated the age-related decrease of blood GSH. Other studies also reported positive relations between blood GSH concentrations and the amount of daily physical activity performed [41] or the aerobic training level [29,43]. Moreover, in elderly subjects (aged between 60 and 83 years), plasma total protein and non-protein (GSH) thiols were higher after a resistance training exercise program [44].

Indeed, we observed lower plasma total protein -SH groups in EE subjects as compared with their younger counterparts. A decrease in plasma total protein -SH groups is known to be induced by a wide array of reactive oxygen species and is one of the most immediate responses to oxidative stress. Functional consequences of -SH group oxidation include protein misfolding, catalytic inactivation, decreased antioxidant capacity and loss of some specific functions [45]. It is known that Zn prevents the reaction between thiols and iron [46]. As EE subjects also had a lower but not significant Zn status as compared with their younger counterparts and because total -SH group and plasma Zn concentrations tended to be positively correlated in EE subjects, it can be postulated that the low total -SH group concentration observed in EE subjects is partly explained by their Zn status.

Subjects were living in the South of France. With regard to their dietary habits, elderly subjects adopted dietary characteristics typical of a Mediterranean diet mostly, as was previously shown by Schröder et al. [47] in a representative Mediterranean population of Spain. In contrast to their younger counterparts, the quality of macronutrient intakes in EE subjects was not different from that in sedentary subjects even with the increase in energy intake. As a consequence, EE subjects had higher β-carotene, vitamin C and vitamin E intakes as compared with EY subjects. Dietary intakes allowed EE subjects to preserve the nutrient density of their diet and, therefore, reach the FRDA for most antioxidant micronutrients [15]. For vitamin E, intakes were lower than the 2:3 of the FRDAa for 77% of EE subjects. However, none of those subjects has a low plasma α-tocopherol concentration. The FRDAa set for vitamin E with reference to physical activity (12 mg vitamin E/day + 12 mg/day per additional 4.18 MJ energy expenditure >9.21 MJ) may be too high. Nevertheless, it remains unknown whether vitamin E uptake by skeletal muscle and turnover are increased with physical activity with advancing age. Concentrations could depend on plasma HDL and LDL concentrations. Indeed, HDL is known to be the main α-tocopherol plasma transport protein and an excellent vector of its cellular incorporation because of its recognition by specific receptors [48]. In our study, only LDL concentrations differed between groups. A large body of experimental research indicates a protective role of vitamin E in exercise, but the evidence of an increase in requirements with physical activity is still limited and inconsistent even in elderly subjects.

Se, Zn and Cu dietary intakes were higher than those observed in French institutionalized elderly patients [33]. Obviously, high antioxidant intakes failed to counteract the enhanced free radical production with aging.

Despite higher antioxidant intakes as compared with those of their younger counterparts, EE subjects exhibited lower plasma concentrations, particularly in carotenoids (lycopene, β-carotene and α-carotene). The bioavailability of lycopene has been shown to be impaired in elderly subjects (60–75 years), without any major difference in the bioavailability of β-carotene [49]. In our study, plasma β-carotene concentrations were lower in EE subjects as compared with their EY and SE counterparts. Interestingly, this was shown despite significantly higher β-carotene intakes in this group. The consequences of lowered β-carotene status in EE subjects are still unknown, but in epidemiological studies, a low plasma carotenoid status is associated with an increased risk of age-related diseases [50].

In conclusion, high antioxidant vitamin and trace element dietary intakes and exercise training practice in elderly subjects do not balance the age-related increase of oxidative stress. The adaptive mechanism observed in young athletes does not seem to be efficient in elderly athletes. Training and aging effects on GSH-Px activity may not be relevant for the same mechanism. These data strongly suggest specific antioxidant requirements for athletes with advancing age, with a special attention to carotenoids.

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