Exercise, oxidative stress and risk of cardiovascular disease in the elderly. Protective role of antioxidant functional foods

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Abstract. Free radicals and oxidative stress are involved in the pathogenic mechanisms of cardiovascular disease (CVD), diabetes and cancer. Exercise is a useful strategy for preventing CVD but in elderly persons it can enhance oxidative stress, which is why some studies recommend antioxidant supplementation for exercising elderly subjects. This intervention study was performed on 320 elderly subjects following a Geriatric Revitalization Program (GEREPRO) to maintain physical health and reduce CVD risk. GEREPRO was based on regular exercise concurrent with a nutritional antioxidant treatment based on daily intake of a functional antioxidant food, Biofrutas\textsuperscript{TM}. Sustained exercise (10 months, 3 sessions/week) significantly increased cardiorespiratory fitness and plasma HDL-cholesterol; it reduced some predictors of cardiovascular risk (arterial pressure, LDL-cholesterol, total cholesterol/LDL-C, LDL-C/HDL-C), but significantly enhanced some biomarkers of oxidative stress. Concurrent antioxidant supplementation did not produce any ergogenic effects but, meaningfully, enhanced some positive effects of exercise on physical health and the CVD risk index, and it totally prevented the exercise-induced oxidative stress. Our results show that regular and moderate exercise improves cardiorespiratory function and reduces CVD risk in elderly people, while concurrent antioxidant supplementation modulates oxidative insult during exercise in the elderly and enhances the beneficial effects of exercise.

Keywords: Antioxidant vitamins, cardiovascular disease, elderly, exercise, functional antioxidant foods, lipids profile, oxidative stress, VO\textsubscript{2}max

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1. Introduction

Exercise and diet influence health. The reports of the WHO (57th WHO Assembly, May 2004), ACSM [3] and other studies [14, 38] indicate that physical inactivity and unhealthy diets are among the leading causes of the three major non-communicable diseases: CVD, diabetes, and cancer. CVDs are a leading cause of morbidity and mortality [11], and there is substantial evidence pointing to atherosclerosis as being the main causal event in most CVDs [1, 9, 55]. The information currently available links atherosclerosis with oxidized LDL-cholesterol as the compound mainly responsible for its appearance.

Whereas physical inactivity can lead to illness, especially in the elderly, regular exercise is one of the most useful strategies for preventing CVD and is recommended as a cornerstone for the prevention and treatment of other chronic diseases. Furthermore, even if initiated in later years exercise contributes to high physical functioning and overall health, and can reduce the risk of diabetes, some forms of cancer and other chronic diseases [3, 19, 38]. Regarding exercise and CVD, it is well known that besides increasing cardiorespiratory capacity (VO$_2$max) [31] regular exercise reduces some predictor indices for risk by reducing arterial blood pressure (BP), total cholesterol (TC) and LDL-cholesterol (LDL-C); it also can increase HDL-C levels [16, 31, 46] and improve endothelial function [46, 50].

By contrast, it is well established that strenuous exercise, above a certain load or when performed by unfit or unaccustomed individuals (as is often the case of the elderly), significantly increases oxygen consumption and muscle utilization, thereby increasing the generation of reactive oxygen species (ROS), oxidative stress and oxidative injury to cell macromolecules [18, 32]. It is also well known that atherosclerosis [4, 5], ageing [17, 32], and exercise-induced oxidative damage share oxidative stress as a common underlying mechanism. ROS are continuously generated and also continuously neutralized by a network of antioxidant defenses, thus preventing oxidative stress. The antioxidant network includes repair and antioxidant enzymes, and small molecules with scavenging ability, such as glutathione (GSH), flavonoids, other phytochemicals, and the antioxidant vitamins E, C and A [5, 21, 22, 48].

Although epidemiological research has associated high intakes of antioxidants from fruit and vegetables with a lower incidence of CVD, most clinical studies have been unable to show beneficial effects of antioxidant supplementation on the risk of CVD (for reviews see [1, 9, 55]). However, some other epidemiological studies have clearly documented the benefits of α-tocopherol [49, CHAOS], α-tocopherol and vitamin C [8, SPACE] and vitamin C plus vitamin E [43, ASAP] on the risk of CVD. Furthermore, some intervention studies have reported a similar antioxidant role for these vitamins, since $ex$ $vivo$ LDL resistance to oxidation during dietary vitamin E restriction and supplementation was inversely related to the changes in vitamin E status, and directly associated with those observed in lipid peroxidation [56]. Other studies have indicated that oxidative stress increases the susceptibility of LDL to lipid peroxidation, whereas vitamin E and/or C supplementation significantly decreases LDL oxidation [9]. Significantly, it has been reported that the plasma levels of thiobarbituric reactive substances (TBARS), a marker of lipoperoxidation, are directly related to the levels of oxidized LDL antibodies (OLAB), and that there is a trend towards lower OLAB levels when those of plasma vitamin E and C are optimal [35].

In the elderly, a sedentary lifestyle and a nutritional deficiency of antioxidant vitamins are commonly accompanied by increased ROS-mediated oxidative damage, declines in antioxidant enzyme activity, and a depletion of the antioxidant reservoir of the organism, i.e. vitamins [17, 18, 22]. Moreover, vigorous exercise can lead to enhanced antioxidant depletion and oxidative injury in aged individuals [32]. Consequently, the balance between the beneficial and potentially harmful effects of exercise might be of particular importance in the elderly and hence it is reasonable to conjecture whether the body’s
antioxidant defenses in physically active elderly persons are sufficient to counteract ageing- and exercise-linked ROS formation, or whether exogenous antioxidant supplements are needed to prevent or reduce macromolecules and LDL oxidation.

In sum, since physical training increases oxidative stress, particularly in elderly people, thus increasing the susceptibility of LDL to oxidation and the risk of CVD; antioxidant efficiency declines with age, and antioxidant supplementation may decrease the susceptibility of LDL to oxidation, and hence the risk of CVD. In light of all this, we have tested the hypothesis that long-term, regular and controlled exercise in elderly people, together with concurrent daily antioxidant vitamin supplementation in the form of a functional antioxidant food, Biofrutas™, might improve cardiovascular health by both reducing some CVD risk markers and preventing or palliating exercise-induced oxidative stress and injury to macromolecules.

2. Material and methods

2.1. Study design and subjects

This study was designed as a long-term intervention study and was performed as a randomized and controlled investigation based on both the practice of regular exercise and nutritional antioxidant treatment. The class-based exercises were selected, programmed, supervised, monitored, and evaluated by physiotherapists specialized in elderly subjects. The antioxidant treatment consisted of a long-term controlled daily supplement intake of the antioxidant vitamins E, C and A. The investigation protocol conformed to the principles outlined in the Declaration of Helsinki (1996) for research in humans. Approval for all the experimental procedures was obtained from the Ethics Committee of the University of Salamanca and the Bioethics Board of the University Hospital of Salamanca.

Participation in the study was offered to 532 eligible members of the Social Network of Community Centres, and a cohort of 320 aged persons was selected (234 women and 86 men, 58–86 years old when selected). Thirty exclusion criteria were considered, including the absence of significant pathologic values in blood clinical parameters, and pathologies such as cancer, diabetes mellitus, renal, respiratory and cardiovascular insufficiency, Wolf-Parkinson-White syndrome, ventricular or aortic aneurysm, pulmonary or systemic embolus, chronic cor pulmonale, balance-related pathologies, neuromuscular diseases, serious osteoporosis, morbid obesity … Subjects with visual, auditory and severe psychological handicaps were also excluded. The individuals selected for the study were not obese (BMI < 30 kg/m²) and had not consumed diets intended to cause weight loss within 6 months prior to selection. Thus, all participants were healthy or at least had no acute medical problems; they were self-sufficient, non-institutionalised, with normal dietary habits, and they were able to perform aerobic exercises of moderate intensity safely. The subjects were fully informed about the risks and benefits associated with the study, and those that agreed to participate gave informed written consent to do so. Following this, the 320 subjects selected were randomized to one of two groups: Exercise (EXER) and Exercise plus Antioxidant Treatment (EXERAT). All subjects followed a supervised class-based exercise program delivered over 10 months (3 sessions/week, 50 min/session). Only the subjects in the EXERAT group were given a nutritional treatment, consisting of the daily intake of 330 mL (one tetrapak) of a functional antioxidant drink, Biofrutas™. The major components and their concentrations in Biofrutas™ are as follows: 33.7% of a blend juice fruit (orange, lemon carrot, peach, maracuya, haw, and pineapple), 10% of skimmed milk, vitamins E (1.5 mg/100 g, d-α-tocopherol), C (9 mg/100 g, ascorbic acid) and A (120 µg/100 g, β-carotene), sugar, dextrose, aroma, stabilizer (pectin) and water. The nutritional and
energetic values (per 100 g) are as follows: 0.36 g of protein, 14.2 g of carbohydrates, and 0.07 g of lipid; energy 246 KJ (59 Kcal). The individual packages of Biofrutas\textsuperscript{TM} were delivered weekly and given gratis to each participant. The EXER subjects were given water as a placebo.

Subjects receiving the antioxidant treatment consumed as a supplement a total of 5 mg of vitamin E (100% d-\alpha-tocopherol), 30 mg of vitamin C (ascorbic acid) and 400 \( \mu \text{g} \) of vitamin A (\( \beta \)-carotene) per day, representing between 70%–90% of the RDA for these vitamins (as a function of the vitamin, age, sex, RDA guidelines or institutional recommendations considered) [9].

The exercise sessions were conducted at 12 Social Network Community Centres and were directed by a physiotherapist and an assistant in groups of 20–30 participants per group. To improve fitness, balance and cardiorespiratory capacity, the exercise program included the use of multiple components, according to the key practices recommended by the ACSM [3]. Each workout session was similar in nature and consisted of aerobic exercises, beginning with a 10 min warm-up period (stretching and walking) and finishing with a 5-min cool down and relaxation period. The main part, 35–40 min, consisted of strength, endurance and flexibility/balance training of different intensities. The intensity of exercises was gradually increased but the maximum effort never surpassed 70% of the maximum heart rate (100% of maximum load of effort during exercises = 200 minus age, according to the Worms rule). In the middle and at the end of each session participants took a placebo or Biofrutas\textsuperscript{TM} for adequate hydration, and liquids were given under supervision. Compliance with the experimental protocol (attendance at the exercise sessions and compliance with the daily intake of the individual package of Biofrutas\textsuperscript{TM}) was recorded daily for each participant by the physiotherapists or assistant. A logbook was kept, detailing the content and any relevant observations of each week’s sessions. All participants were instructed to follow their normal diet and not to consume any supplementary antioxidant during the study period. None of the subjects studied performed any kind of specific sport and devoted less than 2 h per week to recreational and other programmed physical activity along the study.

2.2. Assessment schedule, blood sampling and parameters evaluated

Participants were tested (by trained researchers and physiotherapists) on 2 different occasions during the study: before (baseline measurements, at week 0) and after the interventions (at the end of the study, 10 months). Seven-day diet records for comparing nutrient intakes between the groups (Diet Analysis Plus Test) were administered to each participant during the week prior to each assessment.

Blood samples were collected from all subjects before and after the interventions. Blood was collected by venipuncture and placed in vacutainer/siliconized tubes containing a separating gel and EDTA as an anticoagulant agent. Blood samples were collected at a clinical laboratory after an overnight fasting period, at between 8.00–9.00 am; that is, 16–18 hours after the last session of physical activity and/or the last dose of Biofrutas\textsuperscript{TM}. Cardiovascular (BP and resting heart rate) and anthropometric (weight, height, body mass index – weight/height, Kg/m\(^2\)) parameters were also evaluated.

To estimate cardiorespiratory fitness, a one-mile walk test (Rockport Fitness Walking Test – RFWT) was administered to determine maximal oxygen uptake (\( \text{VO}_2\text{max} \)). The RFWT has been validated for elderly people by Kline et al. [25]. The test was administered before and after the interventions under comparable climatic conditions. After a brief warm-up period, the subjects walked one mile as fast as they could. The time taken to walk one mile and heart rate were recorded immediately after the subjects had crossed the finish line, and were used for the estimation of \( \text{VO}_2\text{max} \).
2.3. Plasma biochemical and oxidative stress marker determinations

A blood sample from each subject was centrifuged (4°C, 4000 rpm, 5 min) immediately after sampling and plasma biochemical markers for CDV estimation (total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides) were evaluated by routine methods at the clinical laboratory using an automated autosampler (Hitachi, mod. 717, Boehringer-Ingelheim, Germany). The remaining plasma was stored in 400-µl aliquots in liquid nitrogen until use for determining the concentration of total glutathione (TGSH), TBARS, as an indicator of lipid oxidation, and reactive carbonyl derivatives of proteins (PC), as an indicator of protein oxidation. The plasma samples were stored in multiple aliquots and each sample was thawed only once and immediately analyzed for the extent of lipid peroxidation; this precaution was taken because prior experimentation had indicated that plasma subjects to even a single freeze-thaw cycle showed increases in baseline lipid peroxidation products. The amount of aldehyde products generated by lipid peroxidation was quantified spectrophotometrically by measurement of TBARS levels [39]. The carbonyl group content of plasma proteins was determined spectrophotometrically with 2,4-dinitrophenyl-hydrazine, as described previously [28]. Plasma TGSH was evaluated by an enzymatic procedure, using GSH reductase and 5,5’-dithio-bis (2-nitrobenzoic acid) (DTNB), according to Adams et al. [2]. The 8-hydroxy-2-deoxyguanosine (8-OHdG) content in leukocyte DNA, as an indicator of DNA oxidation, was also evaluated. Total DNA from peripheral leukocytes was extracted and the amount of 8-OHdG was measured following an HPLC method described previously [30]. The 8-OHdG levels were expressed as the number of 8-OHdG molecules per 10⁵ dG.

2.4. Statistical analysis

Statistics and graphics were computed with a software package for Macintosh computers. Data were processed using JMP™, version 5.0, statistical software (SAS Institute, Cary, NC) and are presented as means ± SEM. Descriptive variables were analyzed using an unpaired Student’s t-test. Differences between post- and pre-intervention values were evaluated using the Student t-test for paired values. Differences between groups were determined by the Student t-test for unpaired samples. ANOVA and significant interactions and main effects were further analyzed using Scheffé’s post hoc test for multiple comparisons between independent groups. A p value <0.05 was considered significant.

3. Results

3.1. Participant characteristics and compliance to treatment

The baseline characteristics of the participants are shown in Table 1. Their anthropometric values can be considered within the normal ranges for this kind of population, and there were no statistical differences between the EXER and EXERAT groups in the baseline measurements as regards age, height, weight, or the body mass index of the subjects. Dietary intake, assessed over the week prior to each of the two assessments performed along the study, was consistent with the RDA measured by 24-h dietary descriptions and no significant differences in average calorie intake (between 1800–2000 kcal per day) and the daily consumption of macronutrients (%), vitamins, and minerals (data not shown) were observed, except for the vitamins in the supplemented subjects.

None of the subjects reported adverse effects associated with the exercise practice or with the daily intake of Biofrutas™. Subjects of the EXER and EXERAT groups had average attendances/compliances
Table 1
Baseline descriptive characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Exer</th>
<th>Exerat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>184</td>
<td>140</td>
</tr>
<tr>
<td>Women</td>
<td>131</td>
<td>103</td>
</tr>
<tr>
<td>Men</td>
<td>53</td>
<td>37</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.3 ± 0.4</td>
<td>69.1 ± 0.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.53 ± 0.06</td>
<td>1.54 ± 0.08</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>67.5 ± 0.7</td>
<td>69.6 ± 1.0</td>
</tr>
<tr>
<td>Men</td>
<td>77.3 ± 2.2</td>
<td>78.2 ± 1.5</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>29.3 ± 0.3</td>
<td>29.6 ± 0.3</td>
</tr>
</tbody>
</table>

Values are given as means (±SEM) for exercise (EXER) and exercise plus antioxidant treatment (EXERAT) groups.

Table 2
Baseline values for aerobic capacity and arterial blood pressure. Influence of sex and age

<table>
<thead>
<tr>
<th></th>
<th>VO₂max (mL/Kg/min)</th>
<th>Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Systolic</td>
</tr>
<tr>
<td>Total</td>
<td>21.6 ± 0.7</td>
<td>98.4 ± 0.7</td>
</tr>
<tr>
<td>Women</td>
<td>20.4 ± 0.7</td>
<td>98.6 ± 0.8</td>
</tr>
<tr>
<td>Men</td>
<td>27.2 ± 1.6 W</td>
<td>97.4 ± 1.5</td>
</tr>
<tr>
<td>&lt; 65 years</td>
<td>25.4 ± 1.0</td>
<td>95.6 ± 1.4</td>
</tr>
<tr>
<td>65-69 years</td>
<td>21.5 ± 1.1 I</td>
<td>98.9 ± 1.2</td>
</tr>
<tr>
<td>70-74 years</td>
<td>20.0 ± 1.2 I</td>
<td>99.5 ± 1.3 I</td>
</tr>
<tr>
<td>≥ 75 years</td>
<td>17.9 ± 1.1 I, II</td>
<td>100.0 ± 1.2 I</td>
</tr>
</tbody>
</table>

Values are given as means (±SEM) for the whole study population; n = 304–320; W, I, II: p < 0.05, significantly different from women, <65 years and 65–69 years, respectively.

The effects of exercise and concurrent antioxidant supplementation on VO₂max values of the whole population, estimated by the RWT, are shown in Table 2. Considering all the participants, mean VO₂max levels were similar to those described by other authors for subjects of the same age. After separating by sex, the VO₂max values were significantly higher in men than in women, and this difference was also observed at the end of the study: i.e., after exercising (data not shown). In addition, a progressive decline with age was observed both before (Table 2) and after (data not shown) the subjects had performed the exercise program, the mean values in subjects <65 years old being significantly higher than in the other age groups.

The effects of exercise and concurrent antioxidant supplementation on VO₂max are shown in Fig. 1 (upper panel). It can be observed that exercise induced a significant increase both in the EXER and the
antioxidant-supplemented subjects (+12.6% and +10.1%, respectively), but no significant differences were observed between either experimental groups, nor before or after the interventions. Figure 1 (low panels) shows the exercise-induced increases in VO_{2max} in the whole population (EXER plus EXERAT subjects) separated by sex and age. The beneficial effects of exercise were higher in women (+14.1%) than in men (+6.8%) but were independent of the age of the participants, the percentage increases varying between 11.2% in the oldest (>75 years) and 14.0% in the subjects in the 65–69 year age group.

Table 2 shows the baseline mean values of arterial pressure and Fig. 2 shows the changes in mean, systolic and diastolic arterial pressure before and after the interventions in the EXER and EXERAT subjects. It may be seen that exercise alone elicited significant reductions in mean (−2.6 mmHg), systolic (−3.7 mmHg) and diastolic (−2.1 mmHg) arterial pressure. A similar beneficial effect was observed in the subjects performing the same exercise program but who were also receiving a daily intake of the antioxidant functional food. However, the declines observed in the mean (−4.3 mmHg) and diastolic (−4.0 mmHg) arterial pressure in the subjects receiving antioxidant treatment were significant higher than those observed in the non-supplemented subjects.
Fig. 2. Effects of exercise and exercise plus antioxidant treatment on the arterial pressure. Values (mm Hg) are given as means (±SEM) before and after the physical activity programme. *, e: p < 0.05, significantly different from before and EXER group, respectively; n = 135 to 172.

3.3. Effects of exercise and antioxidant supplementation on the plasma lipid profile

The mean plasma TC, HDL-C, LDL-C, VLDL-C and triglyceride concentrations, and the TC/HDL-C and LDL-C/HDL-C ratios before and after the interventions are shown in Table 3 (absolute values) and in Fig. 3 (as percentage changes relative to baseline values). Under resting conditions – i.e., before the interventions- the mean values of all these parameters were within the normal physiological range. No significant changes were observed either in VLDL-C or triglyceride levels after the interventions. However, both exercise alone and exercise plus concurrent daily intake of the antioxidant functional food caused significant increases in plasma HDL-C levels, to a similar degree. In addition, the TC/HDL-C
and LDL-C/HDL-C ratios were significantly reduced after the interventions, the fall in the latter index being higher in the antioxidant-supplemented (−11.5%) than in the non-supplemented (−8.4%) subjects. After the interventions, LDL-C was slightly decreased only in the EXERAT subjects, and TC was slightly increased only in the EXER subjects; these changes were significant when compared for paired values (pre- vs post-interventions), but not when compared for unpaired values (EXER vs EXERAT) both before and after the interventions.

3.4. Effect of exercise and concurrent daily intake of Biofrutas™ on systemic oxidative stress biomarkers

The effects of exercise and of the daily intake of the antioxidant functional food on the plasma values of TBARS, PC and TGSH, and leukocyte 8-OHdG contents are shown in Table 4 and Fig. 4. The results are depicted as absolute values (Table 4), and as percentage changes relative to pre-intervention values (Fig. 4). It may be seen that the mean TBARS, PC, 8-OHdG and TGSH values before starting the exercise and antioxidant supplementation interventions were not statistically different on comparing the EXER and EXERAT groups. In addition, the mean baseline values of the four makers of oxidative stress of the whole population were higher in the older than in the younger subjects, but the differences were not significant (data not shown).

The results clearly show that the exercise program performed by the subjects of the EXER group significantly increased oxidative stress since the mean values of TBARS, PC, TGSH and 8-OHdG were significantly higher after than before the exercise intervention. The pro-oxidant effects of exercise on lipid and protein levels were related to the degree of attendance; thus, after the end of the exercise

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**Table 3**

Effects of exercise and exercise plus antioxidant treatment on the plasma lipid profile

<table>
<thead>
<tr>
<th></th>
<th>Exer Before</th>
<th>Exer After</th>
<th>Exerat Before</th>
<th>Exerat After</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>206 ± 3</td>
<td>210 ± 3∗</td>
<td>210 ± 3</td>
<td>211 ± 3</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>42.4 ± 0.4</td>
<td>47.8 ± 0.8∗</td>
<td>44.0 ± 0.6</td>
<td>49.0 ± 1.0∗</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>144 ± 4</td>
<td>145 ± 2</td>
<td>148 ± 3</td>
<td>143 ± 2∗</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>20.3 ± 1.6</td>
<td>17.3 ± 0.9</td>
<td>17.9 ± 0.8</td>
<td>18.2 ± 0.8</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>4.86 ± 0.05</td>
<td>4.45 ± 0.06∗</td>
<td>4.79 ± 0.06</td>
<td>4.37 ± 0.07∗</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>3.37 ± 0.09</td>
<td>3.08 ± 0.05∗</td>
<td>3.37 ± 0.05</td>
<td>2.98 ± 0.06∗</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>101.3 ± 7.8</td>
<td>86.4 ± 4.7</td>
<td>89.7 ± 3.8</td>
<td>91.1 ± 3.8</td>
</tr>
</tbody>
</table>

Values are given as means (±SEM) for exercise (EXER) and exercise plus antioxidant treatment (EXERAT) groups, before and after the physical activity programme; n = 136−180; ∗: p < 0.05, significantly different from before.

**Table 4**

Effects of exercise and exercise plus antioxidant treatment on oxidative stress markers

<table>
<thead>
<tr>
<th></th>
<th>Exer Before</th>
<th>Exer After</th>
<th>Exerat Before</th>
<th>Exerat After</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (µM)</td>
<td>2.91 ± 0.07</td>
<td>3.32 ± 0.08∗</td>
<td>3.19 ± 0.08</td>
<td>2.60 ± 0.05∗∗</td>
</tr>
<tr>
<td>Protein Carbonyls (µM)</td>
<td>30.4 ± 0.5</td>
<td>35.6 ± 0.6∗</td>
<td>31.5 ± 0.6</td>
<td>29.3 ± 0.8∗</td>
</tr>
<tr>
<td>8-OHdG (8-oxoG/10^5dG)</td>
<td>29.5 ± 4.3</td>
<td>36.2 ± 2.6∗</td>
<td>29.9 ± 1.9</td>
<td>22.0 ± 1.9∗</td>
</tr>
<tr>
<td>Total glutathione (µM)</td>
<td>2.63 ± 0.09</td>
<td>1.87 ± 0.07∗</td>
<td>2.69 ± 0.09</td>
<td>1.99 ± 0.09</td>
</tr>
</tbody>
</table>

Values are given as means (±SEM) for exercise (EXER) and exercise plus antioxidant treatment (EXERAT) groups, before and after the physical activity programme; n = 136−180; ∗∗: p < 0.05, significantly different from before and EXER group, respectively.
sessions, the mean plasma TBARS concentrations in subjects who attended \( \geq 90\% \) of the sessions were significantly \(( p < 0.05)\) higher \((+24\%)\) than those who attended \( \geq 75\% \) of the sessions \((+14\%)\). In the case of PC, these changes were +26\% and +17\%, respectively \(( p < 0.01)\). The results on the oxidation of peripheral leukocyte DNA also show that sustained exercise increased 8-OHdG concentrations in the EXER subjects, the mean values after exercise being significantly higher than the pre-exercise values.

By contrast, in the EXERAT group, -i.e., when the exercising subjects were supplemented with antioxidants-, the daily intake of Biofrutas\textsuperscript{TM} completely prevented the pro-oxidant effects of exercise, except in the case of TGSH. It may be seen (Table 4) that the post-intervention mean values of TBARS, PC and 8-OHdG were significantly lower than those obtained before the start of the exercise sessions and concurrent intake of Biofrutas\textsuperscript{TM}, with decreases in TBARS, PC and 8-OHdG of about 20\%, 10\% and 20\%, respectively with respect to the pre-exercise values (Table 4 and Fig. 4). In addition, the mean values at the end of the interventions were significantly lower in the EXERAT than those observed in the non-supplemented subjects. The daily intake of the antioxidant functional food was totally ineffective at preventing the exercise-induced depletion in plasma TGSH since its mean values after the intervention were similar in the EXER and EXERAT subjects.

These results on the major oxidative stress markers clearly show that sustained exercise, even at moder-
Fig. 4. Effects of exercise and exercise plus antioxidant treatment on the oxidative stress markers. Values are given as means (±SEM) for EXER (■) and EXERAT (□) groups. Results correspond to the data obtained after the physical activity programme, and are expressed as percentage changes with respect to baseline values; n = 136 to 175 *: p < 0.05, significantly different from before.

4. Discussion

Conventional risk factors such as hypertension, obesity, diabetes, hypercholesterolemia and high LDL-C levels, hypertriglyceridemia, a fat-loaded diet, smoking, alcohol and low physical activity, among others, are known to contribute to varying extents to the incidence and progression of atherosclerosis and CVDs, and are responsible for >80% of cardiovascular problems [9,55]. Exercise is considered to be the cornerstone for the prevention and treatment of CVD, but it is well known that exercise above a certain load, or when it is performed by unfit individuals, increases ROS generation, oxidative stress and lipoperoxidation [18,32], and these redox disturbances could enhance LDL oxidation, thus contributing/initiating the atherosclerotic process [4,14]. Additionally, the amount and type of physical activity that should be recommended to bring about positive effects on cardiovascular health is unclear, and the positive effects of physical activity on certain cardiac risk factors are still under debate. Also, the beneficial effects of concurrent antioxidant supplementation to protect against exercise-induced oxidative stress and to improve the main CVD risk index in physically active old people have not been completely elucidated. Our research demonstrates that besides increasing cardiorespiratory fitness in men and women the long-term aerobic exercise program administered to elderly people significantly improved some predictors of CVD risk; when the antioxidant food was administered daily and concurrently no ergogenic effects were detected, but additional beneficial effects were observed in oxidative stress status,
mean BP and the LDL-C plasma levels, which were significantly lower than those observed in the non-supplemented subjects.

The mechanisms by which physical activity positively influences the risk of CVD remain poorly understood, although it has been widely demonstrated that regular exercise, even if initiated in later years, increases work efficiency, performance and cardiorespiratory fitness. The latter is one of the main indicators of the functional state of individuals, and one of the main determining factors of physical health in aged people [3,31]. We observed that the moderate-intensity exercise program applied in our study significantly increased the cardiorespiratory capacity in women and men regardless of their adscription to the EXER or EXERA T groups. This clearly demonstrates that the concurrent antioxidant intervention in the latter group did not modify, either positively or negatively, the beneficial effect derived from exercise on cardiorespiratory capacity. A similar absence of ergogenic effects has been reported previously after antioxidant supplementation in both trained and untrained persons of different ages and levels of fitness [42,53]. The improvement observed in VO\textsubscript{2max} partially agrees with that reported by Hawkins and Wiswell [18] in a long-term program. These authors reported that exercise, if maintained long-term, may reduce -in an age-related manner- the VO\textsubscript{2max} up to 50% in young and middle-aged men, but not in older men [18]. By contrast, our results are in total agreement with other studies reporting a similar degree of improvement in VO\textsubscript{2max} after both short-term (≤16 weeks) [33] and long-term training periods (>20 weeks) [15], after moderate or high-volume and frequency of training (3–6 times per week), and even during a short-term training period [20,26], possibly due to a synergistic positive effect of strength and endurance training. Finally, as regards amount, the increases observed by us in VO\textsubscript{2max} are similar to those reported in recent studies carried out on active aged adult individuals [13, 20] and in subjects over 80 years of age [40]. After separating the subject sample by sex and age, we observed a greater improvement in women than in men, and in 65 to 70-year-old subjects than in older ones (>75 y). These observations are in accordance with previous studies reporting age- and sex-related differences in VO\textsubscript{2max}, since current evidence supports a 5–20% per decade decline in cardiorespiratory fitness in men and women regardless of the activity level and sex [3,18]. This seems to be due to both central and peripheral adaptations, primarily reductions in maximal heart rate and lean body mass, a progressive reduction in muscle mass, and a reduced capacity for oxygen transport [3,18,31].

Most epidemiological studies have reported that an active lifestyle and increased physical activity is associated with reduced arterial pressure [46,47]. Our results on BP clearly demonstrate that the exercise program significantly reduced systolic, diastolic and mean BP, the amount of decline being similar to those observed in elderly following a similar program [7,20]. Cook et al. [10] have demonstrated that a reduction in BP by about 2 mmHg resulted in a 17% reduction in the prevalence of hypertension. Similarly, decreases in the resting heart rate and BP concomitant with increases in the VO\textsubscript{2max} have been reported in healthy elderly men performing regular aerobic training in endurance disciplines (e.g. walking, jogging or cycling) [20]. Finally, in a recent meta-analysis on the effect of exercise on BP Whelton et al. [54] concluded that training in aerobic exercise is associated with a significant reduction in systolic and diastolic pressure (−3.35 and −2.58 mmHg, respectively), and these declines have been observed in both normotensive and hypertensive elderly subjects as well as in normal-weight and overweight participants. Although our research does not allow to us to unravel the mechanism(s) involved in the exercise-induced VO\textsubscript{2max} increase and reduction of BP, emerging evidence suggests that exercise improves heart and endothelial function. Thus, the beneficial effects for BP and VO\textsubscript{2max} observed by us might be associated with exercise-related improvements in left ventricular structure and function possibly due to decreased left ventricular mass and wall thickness [7,20]. In addition, peripheral health, such as a reduced stiffness of the arterial vessel walls and increased capillarization within the exercising muscle groups.
in older trained subjects have been reported [7,20]; more, an improvement in endothelium-dependent vasodilatation involving nitric oxide has been also shown in men [46,50].

Regarding the effects of exercise on plasma lipid profile there is considerable variability in the results of exercise/lipid-lowering studies, in part because of the heterogeneity of the study methods, the populations examined or the exercise interventions. Most exercise studies have demonstrated that endurance-trained individuals are characterized by a lower atherogenic lipid profile [16,27,31,46]. The data available generally support the conclusion that older adults who follow a structured exercise programme improve their plasma lipoprotein lipid profiles [31]. The most commonly observed changes in elderly people are an increase in HDL-C and a decrease in the TC/HDL-C ratio, with or without concomitant reductions in TC and LDL-C, and reductions in triglycerides, although these are less frequently observed [27,31]. Small but significant HDL-C increases have been observed after two-year exercise training in 50- to 65-year old men and women, with no significant effects on TC, LDL-C, and TG levels [24]. Also, HDL-C, LDL-C, TC and triglycerides tended to change in a favourable direction but not in a significant manner after six months of supervised aerobic training in 229 elderly men and women [44]. A meta-analysis of 95 studies, most of which were not randomized controlled trials, concluded that exercise leads to a 6.3% reduction in total cholesterol; 10.1% in LDL-C; 13.4% in TC/HDL-C, and at least a 5% increase in HDL-C [52]. Overall, our results are in agreement with those described above, but they also demonstrate that exercise training in conjunction with antioxidant supplementation is somewhat more effective than exercise alone in reducing the risk of CVD since BP, plasma LDL-C levels and the LDL-C/HDL-C ratio were more reduced than in the non-supplemented individuals. Similar findings have been reported in vitamin E-supplemented trained rats of different ages [6], but at present there are no data on the lipid profile from long-term and large-scale intervention studies in healthy elderly people based on both exercise and antioxidant supplementation with a functional antioxidant food.

Regarding the effects of nutritional treatment, we observed that apart from preventing exercise-induced oxidative stress the simultaneous exercise and antioxidant supplement intervention significantly enhanced the beneficial effects on CVD risk caused by exercise alone, suggesting a certain synergic effect of training and antioxidants in cardiovascular health. A large number of epidemiological (MONICA, NHS, Basel, HPFS, Edinburgh...) and prospective randomized intervention (ATBC, CHAOS, SPACE, GISSI,...) studies focused on CVDs have concluded that high intake levels of antioxidant vitamins from either foods or supplements efficiently prevent and reduce CVDs [8,9,43,49]. The mechanism responsible for this preventive effect that has been most studied is their antioxidant capacity, and some authors have strongly suggested that exposure to a diet rich in antioxidant vitamins significantly improves the antioxidant capacity of blood and tissues [9,55,56], thus helping to protect against ROS-induced oxidative damage to macromolecules, i.e. LDLs, proteins and DNA. Nevertheless, as reviewed recently [1,9,37,55], the vast majority of large interventional studies on antioxidant vitamins have shown no reduction in coronary events or even any adverse effects. However, some interventional studies have also reported clear beneficial effects of antioxidants on LDL-C oxidation and atherosclerosis (see Introduction). In this sense, it has recently been reported that moderate exercise in hypercholesterolemic mice induced systemic oxidative stress and reduced atherosclerotic lesions in comparison with sedentary control mice; in addition, antioxidant vitamin supplementation to those exercising mice further and synergistically reduced some CVDs risk indicators and atherosclerotic lesions as compared with untreated exercised mice [36]. Also, significant decreases in the susceptibility of VLDL-C and LDL-C to in vitro oxidation, and increases in total peroxyl-radical trapping were observed by Kaikkonen et al. [23] in runners previously supplemented with vitamin E and coenzyme Q10 before a marathon. Moreover, it has recently been reported that oxidized LDL antibodies (OLAB) levels are directly related to plasma
TBARS concentrations in both young and elderly humans, and both OLAB and TBARS are inversely related to vitamin C and vitamin E intake from either foods or supplements [35]. Our results on TBARS, PC and 8-OHdG levels and CVD risk indices are completely compatible with all the above findings since preliminary data on plasma antioxidant vitamins reflect higher plasma levels in the supplemented than in the EXER subjects. Finally, in one of the most recent interventional large-scale studies (the VITAGE study group) [56] in 100 healthy non-smoking 20–75-y old subjects under dietary vitamin E depletion/supplementation it was observed that changes in the vitamin E status were associated with decreases in lipid peroxidation and oxidative DNA modification during the supplementation period, and with decreases and increases, respectively, in LDL resistance to oxidation in the depletion and supplementation period. These results are in agreement with other studies [9], and clearly suggest both that oxidative stress increases the susceptibility of LDL to lipid peroxidation, and that vitamin E supplementation significantly decreases both this and the amount of oxidized LDL, thus helping to reduce the risk of CVDs. In this sense, it is now well known that vitamin E and some carotenoids are integrated in LDLs and protect them very efficiently against ROS-induced oxidation. It has also been observed that LDL oxidation is preceded by the successive destruction of these intrinsic antioxidants, particularly vitamin E, which acts as a first line of defense before carotenoids intervene, mainly because its concentration in LDLs is 6 to 8 mol/mol of LDL, whereas those of β-carotene are about 20 times lower [9, 12]. Additionally, besides preventing the oxidation of LDLs, vitamin E may help to prevent CVD, in part by decreasing the expression of proinflammatory cytokines by endothelial cells [34], since it inhibits the production of inflammatory mediators in monocytes, and monocyte adhesion to endothelial cells in vitro [57], and it preserves endothelial function in vitro and in vivo [41]. Likewise, vitamin C seems to improve coronary microcirculation [36] and endothelial vasodilatation [51], and it delays a variety of pro-inflammatory mechanisms in vitro, and in several in vivo models [1,29]. Consequently, antioxidant vitamins appear to exert a variety of important roles in maintaining cardiovascular function and health, ranging from protecting LDLs against oxidation, enhancing the antioxidant capacity of lipoproteins, improving capillary circulation and preserving endothelial tissue [45], and all these phenomena should protect subjects from arteriosclerosis. Some such phenomena might be involved in the beneficial effects observed by us on BP and VO$_2$max in the subjects following the exercise program and jointly receiving the antioxidant vitamin supplementation. Finally, besides antioxidant vitamins Biofrutas™ contains small amounts of a variety of tropical fruit juice extracts that are known to contain significant quantities of antioxidant phytochemicals [55], which could also be involved in the effects observed in this study.

In sum, taken together our results and those of all the above studies clearly suggest that a metabolic intervention based on antioxidant vitamins concurrent with exercise of moderate intensity not only prevents the oxidative stress linked to exercise but also is able to help reduce the risk of CVD in humans. In addition, our data also show that although the concurrent antioxidant daily supplement did not produce any ergogenic effects, it efficiently prevented exercise-induced oxidative stress and macromolecular oxidative injury. These improvements suggest that for elderly people who exercise regularly the daily intake of an antioxidant functional food or a vitamin supplementation might be necessary to preserve redox homeostasis and to maintain a higher degree of functional fitness and cardiovascular health.

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