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Chronic training increases blood oxidative damage but promotes health in elderly men

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Abstract The objective of the present study was to investigate a large panel of oxidative stress biomarkers in long-term trained elderly men to analyse the effects of chronic training on an aged population. We collected blood samples from two groups of male volunteers older than 65 years who maintain a measure of functional independence: one group of sedentary subjects without a history of regular physical activity and the other of subjects who have sustained training, starting during middle age (mean training time=49±8 years). We studied morbidity and polypharmacy, as well as haematological parameters including red cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, red cell distribution width and several oxidative biomarkers including protein carbonyl content and lipid peroxidation in plasma and erythrocytes, red blood cell H_2O_2-induced haemolysis test, plasma total antioxidant activity and the main antioxidant enzymes of erythrocytes: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-*S*-transferase. After adjusting for confounding factors, we observed an increase in all oxidative damage biomarkers in the plasma and erythrocytes of the long-term exercise group. However, we reported a decrease in the number of diseases per subject with statistical differences nearly significant ($p=0.061$), reduced intake of medications per subject and lower levels of red cell distribution width in the chronic exercise group. These results indicate that chronic exercise from middle age to old age increases oxidative damage; however, chronic exercise appears to be an effective strategy to attenuate the age-related decline in the elderly.

Keywords Aging · Long-term training · Oxidative stress · Oxidative damage · Red blood cell distribution width

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Introduction

One of the possible strategies that experts propose to attenuate or slow age-related decline is exercise. Physical activity is a well-known, safe strategy to significantly improve the quality of life of the elderly. There is strong evidence that regular exercise can minimise the deleterious effects of the modern sedentary lifestyle and limit the development and progression of chronic disease and frailty (Westerterp and Meijer 2001; Ji et al. 2010), as long as it is practiced according to the current exercise guidelines (Chodzko-Zajko et al. 2009). Unfortunately, the mechanisms behind the favourable effects of exercise are not fully understood. It is known that regular and moderate physical activity is associated with an increase in the production of reactive oxygen species (ROS), which activates healthy responses (Eskurza et al. 2004; Radák et al. 2008). However, it has long been suspected that aging attenuates the ability of an organism to adapt to oxidative stress due to alterations in cellular structure and signal transduction capacity (Ji 2008). Because a single bout of exercise induces the generation of ROS that can result in oxidative damage to cellular constituents and the elderly are already at risk of oxidative stress (Powers and Lennon 1999; Ji 2001), additional exposure to exercise-related ROS may increase the deleterious imbalance between oxidants and antioxidants. In some studies, training prevents or has no affect oxidative damage (Aldred and Rohalu 2011; Traustadóttir et al. 2011), whereas others have found evidence of an increase in oxidative damage level in older adults that do exercise regularly (Mergener et al. 2009). Furthermore, publications have reported antioxidant defence to either increase, decrease or to exhibit no change in physically active compared to less active older adults (Meijer et al. 2001, 2002; Rousseau et al. 2006; Traustadóttir et al. 2011). Thus, it remains unclear whether regular physical activity favourably affects the redox state of elderly subjects (Wray et al. 2011).

There is little empirical evidence for the role of regular physical activity from middle to old age. Literature about long-term training (e.g. decades) is scarce, limiting the understanding of the influence of physical activity on late in life. The duration of the training protocol alters the metabolic, immune and endocrine consequences of exercise (Urso et al. 2009); therefore, it seems necessary the study of long-term trained elderly subjects. Indeed, Bar-Shai et al. (2008) proposed that if physical training is initiated prior to a critical physiological threshold in younger rodents, it may prove beneficial for the muscles and bones of older animals, but not in sedentary animals or short-term trained elderly animals. Moreover, a recent population-based cohort study of 2,205 men with follow-up over 35 years concluded that there is a graded reduction in the total mortality risk at old ages with increasing physical activity level during middle age (Byberg et al. 2009). On basis of the role of oxidative stress in age-related decline (Cesari et al. 2006; Howard et al. 2007), these investigations strengthened the importance of the analysis of redox balance in elderly humans who started training during middle age.

Therefore, the purpose of this study was to determine the degree of oxidative damage on biological macromolecules and the antioxidant defence system status in the circulation of elderly men who have sustained training since middle age. The study of several oxidative factors and haematological parameters in this group enabled us to investigate the effects of long-term exercise on oxidative stress in the elderly.

Methods

Participants

The study included two groups of men older than 65 years who maintain a measure of functional independence: one group of 13 sedentary subjects without a history of regular physical activity (SE group) and one group of 13 subjects undergoing long-term training who started training during middle age (TE group). To form the two groups, 13 community-dwelling men over 65 years who practiced regular physical activity were recruited from the Sports Service of Oviedo University (Oviedo, Spain), and 29 institutionalised subjects from Santa Teresa nursing home (Oviedo, Spain) were screened for inclusion. Initial evaluations were performed by experienced geriatricians and sport clinicians and included a medical and pharmacology history review, a physical examination and a questionnaire to determine the characteristics of training. The questionnaire required that the participant provides data on past and current physical activities.

Diseases included in the current analysis were cognitive impairment, dementia, osteoporosis, hypertension, chronic obstructive pulmonary disease, depression, heart failure, ischemic heart disease, rheumatoid arthritis, cancer, liver disease, hyperthyroidism, dyslipidemia and diabetes. Only subjects with a Barthel Index (BI) above 85 (functional independence) were enrolled. BI is a widely used tool for the assessment of a patient's level of independence during the basic activities of daily life (Tiainen et al. 2010). BI is a ten-item scale with the following items: feeding, grooming, bathing, toilet use, dressing, walking, transfers, climbing stairs, faecal incontinence and urinary incontinence. The highest score is 100 (independence) and the lowest is 0 (total dependence) (Mahoney and Barthel 1965). Exclusion criteria were recent or current infection, malignant disease, malnutrition and pharmacological interference (steroids, non-steroidal anti-inflammatory agents, immunosuppressive and anti-neoplastic drugs and testosterone).

All subjects recruited from the Sports Service of Oviedo University satisfied the inclusion and exclusion criteria. These subjects trained chronically (at least 60 min of physical activity per day, 3 days per week for the previous 40 years) and continued practicing regular physical activity at the time of the study; therefore, they were assigned to the TE group. Training was self-directed and combined endurance and resistance activities. No breaks in the training period were reported. Of the participants from Santa Teresa nursing home, 13 subjects satisfied the inclusion and exclusion criteria. These subjects neither had a history of regular physical activity nor practiced regular exercise at the time of the study; therefore, they were assigned to the SE group.

All participants received information about the purposes and objectives of the study and signed informed consent documents. The study was approved by the Hospital Central de Asturias (Oviedo, Spain) ethics committee.

Blood collection

All blood samples were obtained in the morning by venipuncture, after a night in fast and having a rest of 15 min, to be sure that blood sample was obtained without previous physical activity. To analyse the usual oxidative stress levels in the TE group, blood was collected at least 24 h subsequent to the last exercise session. Following centrifugation of blood (3,000 rpm, 15 min, 4°C), the plasma was divided into aliquots and stored at −20°C pending analysis. The buffy coat was discarded, and erythrocytes were washed three times with ice-cold isotonic NaCl solution (0.9%), followed by centrifugation (4,000 rpm, 10 min, 4°C). Haemolysis of the washed packed cells was achieved by mixing with cold distilled water. The prepared haemolysates were stored at −20°C pending analysis. Erythrocyte membranes were prepared according to the method developed by Dodge et al. (1963) and stored at −80°C.

Biochemical analysis

The haematological parameters analysed, including red cell count (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV) and red cell distribution width (RDW), were measured using an automated haematology analyser SYSMEX SF-3000 (GMI Inc., MI, USA). Plasma and erythrocyte membrane proteins were measured using the Bradford (1976) method. Protein oxidative damage, measured as protein carbonyls, was determined using the method developed by Levine et al. (1990) with modifications as described by Coto-Montes and Hardeland (1999). Data are presented as nanomoles protein carbonyl per milligram protein for both plasma (pPCO) and erythrocyte membrane proteins (ePCO). Lipid peroxidation was measured by determining the levels of the reactive aldehydes malondialdehyde (MDA) and 4-hydroxy-2-(*E*)-nonenal (4-HNE), the end products of the lipid peroxidation cascade. The amounts of MDA plus 4-HNE were determined in the plasma and erythrocytes using an LPO Assay Kit from Calbiochem (No. 437634), based on the condensation reaction of the chromogene 1-methyl-2-phenylindole with either MDA or 4-HNE. The results are expressed as micromoles (MDA+4-HNE) per gram protein for plasma (pLPO) and micromoles (MDA+4-HNE) per gram haemoglobin (Hb) for erythrocytes (eLPO).

To study in vitro resistance of erythrocytes to ROS, we performed the erythrocyte haemolysis test (HT) using a modified technique of that described by Farrell et al. (1977) and de Gonzalo-Calvo et al. (2011b). Plasma total antioxidant activity (pTAA) was determined using the ABTS/H_20_2 /HRP method modified for plasma samples (Arnao et al. 2001; de Gonzalo-Calvo et al. 2010). The

results are expressed in equivalents of milligrams Trolox per milligram protein.

Erythrocyte superoxide dismutase activity (eSOD; EC 1.15.1.1) was measured according to a protocol developed by Martin et al. (1987). This enzyme inhibits the haematoxylin auto-oxidation to the coloured compound haematein. The results were expressed as SOD units per milligram Hb. Catalase activity (eCAT; EC 1.11.1.6) was assayed according to the method of Lubinsky and Bewley (1979) using H_2O_2 as the substrate. Data are expressed as micromoles H_2O_2 per milligram Hb minute. Glutathione peroxidase (eGPx; EC 1.11.1.9) catalyses the reduction of hydrogen peroxide in the presence of reduced glutathione (GSH). Glutathione reductase (eGR; EC 1.6.4.2) catalyses the reduction of the oxidised glutathione to GSH using $NADPH+H^+$ as a substrate. The assay of GPx and GR was performed by monitoring the oxidation of NADPH as described by Wheeler et al. (1990). Data are expressed as micromoles NADPH per gram Hb minute. Glutathione-*S*-transferase activity (eGST; EC 2.5.1.18) was assayed as recommended by Habig et al. (1974). Data are expressed as micromoles per gram Hb minute.

Statistical analysis

Variables are expressed as the mean and the standard deviation of the mean (SD). The statistical software package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was employed for all statistical analyses. The normality of the data was analysed using the Kolmogorov–Smirnov test. Data were analysed by analysis of variance. All data presented were adjusted with age and functional status as covariates. Potential contributions from morbid conditions were not considered in the analyses. Because this study was designed to evaluate the associations between the oxidative and haematological biomarkers and long-term training in elderly men, it was not intended to adjust for disease conditions. Differences were considered statistically significant when $p<0.05$.

Results

Characteristics of the study subjects

Table 1 summarises the demographic, clinical and training characteristics of the study groups. Thirteen sedentary and 13 chronically trained subjects completed the study. The mean age was 79±4 years for the sedentary group and 74±5 years for the chronically trained group. The functional independence grade of group SE was elevated (BI=94±6), while all participants of the group TE showed total independence (BI=100). Only the TE group was involved in long-term training, with a mean of four training sessions per week, each training session lasted 83 min a day, for the previous 49 years. After adjusting for confounders, age and functional status, the number of medications taken per subject was lower in the TE group ($p<0.001$). Long-term training was also associated with lower number of medical diagnoses per subject than the SE group, with statistical differences nearly significant ($p=0.061$).

Haematological parameters

RDW (Table 2; $p=0.018$) and MCV (Table 2; $p=0.017$) were significantly lower in the TE group than in the SE group. There was no statistical difference in the haematological parameters RBC, HGB or HCT between the SE and TE groups.

Oxidative damage biomarkers

Analysis of the data revealed that pPCO levels were significantly higher in TE group than in the SE group (Table 3; $p=0.003$). When the TE group was compared to the SE group, a significant increase in ePCO (Table 3; $p=0.025$) associated with chronic training was also observed. Likewise, eLPO levels were significantly higher in the TE group compared with SE group (Table 3; $p=0.022$). Moreover, an increase in the MDA+4-HNE plasma levels was observed with long-term training in the aged population (Table 3; $p=0.027$). No statistical difference was observed between the groups in HT levels (Table 3).

Antioxidative status biomarkers

The comparison of plasma pTAA levels between both groups did not reveal significant differences (Table 4). eSOD activity levels were significantly higher in the erythrocytes of the TE group compared to the SE group (Table 4; $p=0.047$). A significant increase in eGPx activity level associated with long-term training

Table 1 Characteristics of the study groups

	Sedentary	Chronic training	*p* value
Number	13	13	–
Age (years)	79 (5)	74 (5)	0.004
Barthel Index	94 (6)	100 (0)	0.007
Total number of diagnoses per subject	1.4 (1.3)	0.3 (0.5)	0.061
Total number of medications per subject	5.0 (1.9)	0.6 (0.7)	<0.001
Mean training practice time (years)	–	49 (8)	–
Mean training frequency (sessions/week)	–	4 (1)	–
Mean training load (minutes/session)	–	83 (30)	–

All data presented as means (SD)

was also observed (Table 4; p=0.033). There was no change in eCAT, eGR or eGST activities associated with chronic exercise (Table 4).

Discussion

This study was designed to investigate the impact of long-term training on oxidative stress in elderly men. We analysed the principal oxidative blood biomarkers in two groups of men older than 65 years who maintained a degree of functional independence, consisting of one group of sedentary subjects without a history of regular physical activity and one group of subjects undergoing long-term training who initiated training during middle age. After adjustment for confounders, age and functional status, our results demonstrated that long-term training increases the macromolecular oxidative damage of both plasma and erythrocytes. However, chronic exercised is associated with reduced medication intake per subject and decreased number of diseases per subject with statistical differences nearly significant (p=0.061), both conditions associated with a better quality of life. Moreover, exercised subjects presented lower RDW values, a strong indicator of all-cause mortality in elderly population (Patel et al. 2010). Taken together, our results suggest that long-term exercise training induces oxidative damage but also offers protection against adverse outcomes in elderly men.

It has been proposed that the search for oxidative damage due to exercise should ideally involve examination of several indices of oxidative damage (Nikolaidis and Jamurtas 2009). Therefore, the level of oxidative damage in our study was assessed by measuring carbonyl protein content and the MDA+4-HNE concentration in plasma and erythrocytes. We observed a significant increase in pPCO in the TE group compared with their sedentary counterparts. A further indication of the oxidative damage associated with chronic training was the significantly elevated pLPO levels in the TE group. Our findings are in accordance with similar studies showing that protein and lipid plasma oxidative damage are elevated in more active elderly subjects (Rousseau et al. 2006; Mergener et al. 2009). Although the role of carbonyl content as biomarker

Table 2 Haematological parameters in sedentary and long-term trained elderly men

	Sedentary	Chronic training	*p* value
RBC ($\times 10^6/\mu l$)	4.8 (0.6)	5.1 (0.2)	0.190
HGB (g/dl)	15.2 (1.3)	15.4 (1.0)	0.822
HCT (%)	44.6 (4.0)	45.0 (2.8)	0.608
MCV (fl)	91.9 (6.3)	89.8 (2.2)	0.017
RDW (%)	13.9 (0.5)	13.0 (0.3)	0.018

All data presented as means (SD)

RBC red blood cell count, *HGB* haemoglobin concentration, *HCT* haematocrit, *MCV* mean corpuscular volume, *RDW* red blood cell distribution width

Table 3 Oxidative damage biomarkers in sedentary and long-term trained elderly men

	Sedentary	Chronic training	*p* value
pPCO (nmol/mg protein)	8.0 (1.4)	10.2 (1.0)	0.003
pLPO (μmol/g protein)	0.12 (0.04)	0.16 (0.06)	0.027
ePCO (nmol/mg protein)	9.4 (3.0)	15.1 (3.9)	0.025
eLPO (μmol/g Hb)	4.0 (0.6)	5.9 (1.1)	0.022
HT (%)	12.9 (7.8)	16.0 (5.2)	0.477

All data presented as means (SD)

pPCO plasma protein carbonyl content, *pLPO* plasma lipid peroxidation level, *ePCO* erythrocyte protein carbonyl content, *eLPO* erythrocyte lipid peroxidation level, *HT* erythrocyte H_2O_2-induced haemolysis test

remains controversial (Gil et al. 2006), carbonylation seems to be much more than a simple biological marker (Sharma et al. 2006), and previous investigation showed that carbonyl content could be generated by oxidative as well as non-oxidative mechanisms (Adams et al. 2001); investigations published on this topic in general reported carbonyl content as a relevant oxidative damage marker (Dalle-Donne et al. 2003; Semba et al. 2007a, b; Greilberger et al. 2010). Therefore, our data suggest that long-term training initiated during middle age could be associated with a rise in plasma biomacromolecular oxidative damage in elderly men compared to sedentary men.

We have additionally analysed the main oxidative damage biomarkers in erythrocytes. We observed higher ePCO and eLPO levels in the TE group in comparison with the SE group. This situation should have deleterious consequences for erythrocyte physiology. Because of their limited biosynthetic capacity and repair mechanisms, erythrocytes accumulate molecular modifications whenever they are exposed to oxidative stress (Lamprecht et al. 2004). Protein carbonylation and lipid peroxidation cause the loss of membrane integrity and functionality altering fluidity and increasing rigidity of the membrane (Mohandas and Gallagher 2008). Consequently, we studied the impact of the molecular damage observed on erythrocyte oxidative stress response by analysing the haemolysis percentage in both groups. The data revealed that erythrocytes from the TE group were as resistant to H_2O_2 as erythrocytes from the SE group. This HT result was unexpected based on the oxidative damage biomarkers described above. HT data could be strongly influenced by MCV values. The TE group showed a decline in MCV value relative to the SE group. MCV is a haematological parameter that indicates the average volume of red cells, with lower values representing lower erythrocyte volume. Willekens et al. (2008) demonstrated that red cells presented vesiculation mechanisms. Erythrocyte vesiculation consists of the elimination of membrane patches containing damage molecules, thereby strengthening the viability of circulating erythrocytes and postponing the elimination

Table 4 Antioxidant biomarkers in sedentary and long-term trained elderly men

	Sedentary	Chronic training	*p* value
eSOD (U/mg Hb)	13.3 (2.8)	15.4 (2.1)	0.047
eCAT (μmol/mg Hb min)	168.8 (56.5)	188.5 (8.8)	0.340
eGPx (μmol/g Hb min)	27.8 (3.2)	29.6 (1.0)	0.033
eGR (μmol/g Hb min)	3.7 (1.9)	3.7 (2.0)	0.923
eGST (μmol/g Hb min)	1.9 (0.1)	1.5 (0.5)	0.120
pTAA (mg Trolox/mg protein)	2.3 (0.7)	2.8 (1.0)	0.745

All data presented as mean (SD)

eSOD erythrocyte superoxide dismutase activity, *eCAT* erythrocyte catalase activity, *eGPx* erythrocyte glutathione peroxidase activity, *eGR* erythrocyte glutathione reductase activity, *eGST* erythrocyte glutathione-*S*-transferase activity, *pTAA* plasma total antioxidant activity

of healthy erythrocytes. It seems reasonable to assume that the increase in the molecular damage of erythrocyte may account for the decline in erythrocyte volume and similar HT levels between both study groups. Furthermore, evidence has shown that training increases the rate of erythrocyte production concomitant with accelerated turnover, resulting in the presence of a steady-state population of younger and more resistant to oxidative stress erythrocytes (Smith 1995; Senturk et al. 2005). Based on those findings, the increase in oxidative damage and the maintenance of HT level could coexist in the erythrocytes of the TE group due to vesiculation mechanisms and/or accelerated turnover.

The presence of increased carbonyl proteins and the end-products of lipid peroxidation in erythrocytes reflects conditions of oxidative stress (Nikolaidis and Jamurtas 2009). In view of the presented data, it is conceivable that the erythrocyte antioxidant defence system was overwhelmed. Few studies have investigated the influence of exercise on eSOD, eGPx, eCAT, eGR and eGST activities in elderly populations. This finding is somewhat surprising because antioxidant enzymes work in networks. Cross-sectional measurements of one or two antioxidant enzymes likely do not reflect the true complexity of the process in vivo. The analysis of multiple antioxidant enzymes could provide a reliable general view of antioxidant enzyme status (Romeu et al. 2010). Despite the absence of mitochondria in erythrocytes, ROS are constitutively produced, mainly due to the high O_2 tension in arterial blood and their abundant haem iron content (Cimen 2008). The major source of free radicals in erythrocytes is Hb autoxidation, resulting in the production of $O_2^{\cdot -}$ (Johnson et al. 2005). Cellular metabolism during exercise increases Hb autoxidation and can result in a high $O_2^{\cdot -}$ concentration (Lamprecht et al. 2004). This situation could explain the increase in eSOD activity, the primary enzymatic antioxidant that detoxifies $O_2^{\cdot -}$, observed in TE group. Furthermore, we have also identified higher eGPx levels in chronically trained subjects compared to their sedentary counterparts. Chronic training could be associated with changes in the erythrocyte antioxidant enzyme network through an increase in the activities of eSOD and eGPx. It is interesting to note that these results agree with previous investigations (Covas et al. 2002; Parise et al. 2005; Rousseau et al. 2006). Despite the fact of the oxidative damage observed, the increase in eSOD and eGPx activities could be in line with a hormetic effect by exercise. Some authors have proposed that the effects of increased ROS associated with exercise could be described by a hormesis curve (Goto and Radák 2010; Ji et al. 2010). Under normal training conditions, oxidative stress-related to exercise is followed by rest periods, and as a result, a physiological adaptation takes place during this rest time, including the upregulation of defence against oxidative stress.

We have observed that long-term exercise from middle to old age (mean training period of 49±8 years) is associated with increased levels of the four blood oxidative damage markers studied. Our findings are in contrast with earlier studies in which regular exercise decreases the level of oxidative damage in animal models (Radák et al. 1999, 2001, 2004; Nakamoto et al. 2007; Radák et al. 2009). Cui et al. (2009) have also proposed that modest exercise initiated late in life can have a beneficial effect on lipid oxidation in the cerebellum of male rats. In the same way, recent report proposed that fit older adults had significantly lower levels of urinary markers of oxidative damage that unfit age-matched controls in a sample population without history of chronic disease (Traustadóttir et al. 2011). Interestingly, current results are in accordance with similar studies in animal models that proposed that initiating exercise training in late middle age increases oxidative damage in senescent rats (Thomas et al. 2010a, b). Direct comparisons of most exercise studies are difficult to make (Yu and Chung 2006). The physiological effects of exercise depends on type and intensity of exercise, previous training status, training program, age, gender, health conditions, genetic background, diet, environment and in some cases even the type of biochemical assay used and the tissue studied (Ji 1993; Urso et al. 2009; Kaliman et al. 2011), and it could be conceivable a discrepancy in results between different investigations. It is important to note that investigations based on long-term exercised subjects (mean training period of 49±8 years) are limited; therefore, in light of the present results, this field deserves a more detailed investigation.

Blood was collected at least 24 h subsequent to the last exercise session, and it is possible that the observed increase in damage markers is a reflection of insults from bouts of acute exercise (Powers and Lennon 1999); however, exercised subjects are regularly trained, and the higher concentration of plasma and erythrocyte oxidative damage in long-term trained participants would be the usual oxidative damage

levels in circulation. Present data could generate controversy. Several publications have associated an increase in oxidative damage with disability, frailty and mortality in the elderly (Cesari et al. 2005, 2006; Howard et al. 2007; Dayhoff-Brannigan et al. 2008). Recent reports by our group have additionally demonstrated that elevated plasma and erythrocyte oxidative damage is associated with aging and adverse outcomes, such as hypoxia, in the elderly (de Gonzalo-Calvo et al. 2010, 2011b). There is evidence that regular exercise can minimise the deleterious effects of the modern sedentary lifestyle and can limit the development and progression of chronic disease and frailty (Chodzko-Zajko et al. 2009). Indeed, we have observed a decrease in the number of diseases with statistical differences nearly significant and medications taken by elderly men who have undergone long-term training compared to sedentary ones, supporting the beneficial effects of long-term training. Moreover, all participants of the TE group showed a total independence level (BI=100). Results of present investigation are in line with the dilemma proposed by a recent publication (Brewer 2010). Brewer reported that the free radical theory could not explain why higher levels of oxidative damage occur with exercise, which generally promotes healthy human aging. It is interesting to speculate about the hypothetically harmless role of oxidative damage observed in chronically trained participants. To analysed this hypothesis, together with morbidity and polypharmacy data, we have studied the indicator of health in the aged population RDW (Patel et al. 2009). To our knowledge, this is the first investigation that studied RDW levels in trained elderly populations. We have observed a significant decline in RDW values in the chronically trained group compared with the sedentary one. RDW is a quantitative measure of variability in the size of circulating erythrocytes, with higher values reflecting greater heterogeneity in cell sizes. It has been proposed to be a biological marker that reflects multiple physiological impairments related to aging (Patel et al. 2010). Several studies showed that higher RDW, even within the normal reference range, was strongly associated with increased risk of death and cardiovascular disease events in older adults (Cavusoglu et al. 2009; Patel et al. 2009). A recent meta-analysis based on seven relevant studies of older subjects (11,827 community-dwelling older adults) with varied health and demographic compositions reported that the elevated RDW level is a powerful predictor of mortality in older adults with and without age-associated disease, independent of several risk factors for death in older subjects (Patel et al. 2010). The lower morbidity, polypharmacy and RDW levels in long-term trained group point to chronic exercise, independent of oxidative damage, as an effective strategy to attenuate the age-related decline in the elderly.

Currently, it is understood that ROS are not constitutively harmful particles (Buffenstein et al. 2008; Dickinson and Chang 2011; Ristow and Schmeisser 2011). ROS production during exercise could be required to induce beneficial responses through mechanisms in which redox state plays a central role. Nicklas and Brinkley (2009) proposed that exercise training-induced improvements in age-related inflammatory status could result from the modulation of intracellular signalling pathways that are mediated by ROS. Indeed, recent investigation with the same chronic trained subjects showed that long-term exercise from adulthood to old age was intimately associated with a strong reduction in blood levels of the main disease-, disability-, frailty- and mortality-relevant inflammatory biomarkers (de Gonzalo-Calvo et al. 2011a). We concluded that long-term exercise training from adulthood to old age could be an effective intervention in treating multiple adverse health conditions. While ROS molecules are generated at low rates under resting conditions, the production of these molecules increases transiently during exercise and they play a role in inducing anti-inflammatory defence mechanisms (Scheele et al. 2009). Publications concerning this fact are extensive: Seo et al. (2006) reported that life-long wheel running reduced the age-related activation of the redox sensitive transcription factor nuclear factor κB, one of the most potent inflammatory transcription factors, in the liver of old rats. In fact, low RDW values have been associated with a reduced age-related pro-inflammatory state in elderly populations (Semba et al. 2010). Furthermore, several investigations suggest that regular exercise can minimise the physiological effects of aging and increase active life expectancy by limiting the development and progression of chronic diseases, such as cardiovascular disease (Seals et al. 2008), arterial hypertension (Aizawa et al. 2009), osteoporosis (Guadalupe-Grau et al. 2009), sarcopenia (Viña et al. 2009), chronic obstructive pulmonary disease (Dourado et al. 2006) and certain cancers (McNeely et al. 2010), diseases in which oxidative stress plays a central role (Valko et al. 2007).

Several limitations of this study must be considered. The primary limitation is the sample size due to the difficulty of identifying elderly subject with the training characteristics presented in this study. A population sample ≥26 participants would have been desirable. Second, we sampled blood from sedentary participants who were institutionalised, which may have biased the study results because it is likely that these subjects have higher disease levels (Bonanini et al. 2008). To minimise this effect, we have studied sedentary subjects with a degree of functional independence (BI=94±6). Finally, the information about training is based on a questionnaire and depends on participants recall.

Despite these limitations, the results of this study have important clinical and conceptual implications. We suggest that long-term training is associated with increased biomacromolecular oxidative damage in elderly men. Regardless of the level of oxidative damage, we report that long-term training offers protection against polypharmacy and morbidity. Moreover, long-term exercise from middle to old age is associated with decreased RDW levels, a relevant risk factor for mortality in elderly subjects. The benefits of long-term training probably exceed the detrimental effects caused by oxidative damage. The role of oxidative damage in chronic training is a finding that warrants further investigation.

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