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Lipid peroxidation, antioxidant status and survival in institutionalised elderly: A five-year longitudinal study

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Abstract
Oxidative stress has been related to ageing and risk of death. To determine whether oxidative status was associated with all-cause risk of death we carried out a prospective study in 154 non-smoking Spanish elderly without major illness. Baseline glutathione peroxidase (GPx) and superoxide dismutase (SOD) were analysed in plasma and erythrocytes. α-tocopherol, β-carotene, lycopene and retinol were determined in serum samples and malondialdehyde (MDA), as a lipid peroxidation marker, in plasma. Mean survival time was 4.3 years. A total of 31 death cases (20.1%) occurred during the follow-up. Plasma-MDA predicted mortality independently of all other variables, while erythrocyte-SOD (e-SOD), β-carotene and α-tocopherol were positively associated with survival. α-tocopherol and MDA were revealed as independent predictors in a joint survival model, being the group with low MDA and high α-tocopherol that with the lowest mortality. In conclusion, a higher risk of death was associated with increased lipid peroxidation and lower antioxidant defenses.

Keywords: *Oxidative stress, malondialdehyde, α-tocopherol, antioxidants, aging, mortality*

Abbreviations: *BMI, body mass index; DNA, deoxyribonucleic acid; e-GPx, erythrocyte-glutathione peroxidase; p-GPx, plasma-glutathione peroxidase; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; e-SOD, erythrocyte-superoxide dismutase; p-SOD, plasma-superoxide dismutase*

Introduction

Ageing has become an important social issue and a public health concern. From a scientific point of view, the possibility of improving the health of the elderly or delaying senescence depends ultimately on our understanding of the ageing process itself. Among the many proposed theories, the free radical theory of ageing has been able to provide a theoretical framework that comprises both stochastic and genetic factors [1,2] and, so, it probably constitutes the best approximation so far to a unified theory of ageing [3]. In its general form, it postulates that ageing is the result of free radical-mediated damage to molecules and tissues, which accumulates with advancing age in an exponential manner causing a loss in cellular function and an increase in the chance of disease and death of all organisms [4,5].

In support of the hypothesis, there are some strong evidences (summarized elsewhere [1,3,6,7]) which strengthened the theory and showed promise that supplementation with antioxidants would counterbalance free radical generation and help prevent cellular damage and ageing. But, although, a large number of epidemiological and clinical studies have paid attention to the potential therapeutic utility of antioxidants [8–13], yet long-term epidemiological data is lacking on the association of oxidative stress and mortality in humans [14,15]. Still, we do not know the actual importance of oxidative mechanisms such as lipoperoxidation in predicting the chance of death, either.

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This longitudinal study has been conducted to analyse the association of several antioxidants (glutathione peroxidase (GPx), superoxide dismutase (SOD), α-tocopherol, β-carotene, lycopene and retinol) and malondialdehyde (MDA), a biomarker of lipoperoxidation, on overall mortality in a cohort of non-chronically ill elderly subjects from the North of Spain who were followed up for up to 5 years.

Sujects and methods

Study sample

The analysed sample consisted of 154 non-smoking elderly subjects (67 men and 87 women) who resided in seven nursing homes of Asturias (North of Spain). Mean age was 71.7 ± 7.3 and 74.2 ± 4.8 years, respectively, for men and women (age interval [p_5, p_{95}]:[61.5, 79.8] years). Exclusion criteria for the study were a medical history of cancer, cardiovascular disease, stroke or neurological impairment, being confined to a wheelchair or suffering from a terminal disease. The medical histories of the subjects were provided by the institutions. Those on chronic anti-inflammatory, antidepressant or thyroid hormone medication were excluded from analyses because of its potential interaction with the oxidant/antioxidant balance. None of the participants was taking antioxidant supplements, either. All analyses were performed in subjects with at least 6 months (180 days) of survival time.

Previously to data collection, participants were informed about the methodology and objectives of the study and were asked to give their consent to participate. The study protocol was approved by the Committee on Ethical Research of the Oviedo University Hospital.

Data collection

The analysis presented in this paper is part of a broader epidemiological prospective study on diet, oxidative status and survival that includes variables on macro-, micro-nutrients and alcohol intake collected by means of a semi-quantitative food-frequency questionnaire in a personal interview carried out by a trained dietitian. Methodological issues concerning dietary assessment have been detailed elsewhere [16].

During a personal interview, information was collected on potential confounders: subjects were asked to rate their health as bad, poor, fair, good or excellent, and to declare whether they followed a special diet (low in calories, low in cholesterol or adapted for diabetes or chewing problems). Subjects were also asked whether they practised exercise on a regular basis. Drug consumption was registered by means of the medical histories of the participants.

Height of the participants was obtained to the nearest 1 mm with a stadiometer (Año-Sayol, Barcelona, Spain) by having the subjects barefoot, in an upright position, with the head positioned in the Frankfort plane. Weight was measured with a 100 g precision scale (Seca, Hamburg, Germany) in light clothes. Body mass index (BMI) of the participants was obtained as weight (in kg) divided by square height (in m^2).

Blood measurements

For each subject, 30 ml of whole blood was withdrawn by venipuncture after a 12 h fast and kept cold in dark until processed in the laboratory. Plasma was obtained from heparin-treated tubes by centrifugation at 1000*g* for 15 min at room temperature within the next 2 h following the extraction. To obtain serum samples, blood was collected in non-treated tubes and allowed to clot in the dark at room temperature before centrifugation. The buffy coat was removed and the remaining erythrocytes were drawn from the bottom, washed three times in cold saline solution (9.0 g/l NaCl) and hemolyzed by addition of 1/6 (by vol) of doubly distilled water containing 5 ml/l Triton X-100, followed by vigorous vortex-mixing and storage on ice for 10 min. Membrane-free hemolysate was obtained by centrifugation and hemoglobin concentration was assayed by the cyanometahemoglobin method (Sigma cat. no. 541–2, Sigma Chemical Co., USA).

MDA concentration was measured in plasma with the commercial kit LPO-586 (Byoxytech, Oxis International S.A., France). Samples were deproteinized by adding 65 μl of trichloroacetic acid to 450 μl of plasma (final acid concentration of ~10%) and centrifuged 10 min at ~11,400*g* at 4°C. Two hundred microlitre of supernatant were assayed for MDA, in the presence of hydrochloric acid to prevent interference of 4-hydroxynonenal, with spectrophotometric detection at 586 nm.

Antioxidant enzyme activities of GPx and SOD were determined both in plasma and erythrocytes. Plasma-SOD (p-SOD) and erythrocyte-SOD (e-SOD) were assayed with the SOD-525 kit, and erythrocyte-GPx (e-GPx), with the GPx-340 test, while plasma-GPx (p-GPx) was immunoenzymatically determined with the pl-Gpx-EIA kit. All commercial kits for measurement of antioxidant activities were obtained from Byoxytech (Oxis International, S.A., France).

Serum retinol, α-tocopherol, β-carotene and lycopene concentrations were quantified by HPLC. Prior to analyses, protein was removed from the samples by precipitation with ethanol. From the supernatant, 400 μl were evaporated in a speed-vac-concentrator at room temperature and the residue was resuspended in 100 μl of ethanol/dioxane (1:1), before adding 150 μl of acetonitrile. Extracted samples (100 μl) were injected and eluted in isocratic conditions with 5% water and 95% mixture of acetonitrile/tetrahydrofuran/methanol/water (684:220:68:28) at a flow rate of 1.5 ml/min. Maximum absorbance for each vitamin

was 325 nm for retinol, 293 nm for α-tocopherol, 456 nm for β-carotene and 474 nm for lycopene. Quantification was performed by area integration in chromatogram. Standards used for peak identification and quantification were purchased from Sigma (Sigma Chemical Co., USA).

Plasma albumin, total cholesterol and triacylglicerides were determined by standard methods. The concentrations of retinol, α-tocopherol, β-carotene and lycopene were adjusted for plasma lipids according to the formula [17].

Antioxidant (molar concentration) × (5.6 mmol/l cholesterol + 1.24 mmol/l triglycerides)/(actual cholesterol + triglycerides levels in individual plasma).

Statistical methods

Normality of continuous variables was analysed by Kolmogorov-Smirnov tests. Student's *t*-tests were used to check for significant differences by groups when variables were normally distributed; when not, a non-parametrical Mann–Whitney *U*-test was performed. Contingency χ^2 tests were used to check for differences in the percentage of subjects in the levels of categorical variables between categories. Cox's proportional hazards regression models were built to perform survival analyses, with predictive variables categorized into tertiles. Tests for linear trends across tertiles were performed. Multivariate models included sex (0 = man, 1 = woman), age (years), BMI (kg/m^2), self-perceived health (1 = bad or poor, 2 = fair, 3 = good or excellent), alcohol consumption (0 = no, 1 = yes), practice of daily exercise (0 = no, 1 = yes), diabetes (0 = no, 1 = yes), use of antihypertensive medication (0 = no, 1 = yes) and plasma albumin concentration (g/l) as covariates, since all these factors have been related with mortality or oxidative status. Kaplan–Meier survival curves were plotted for comparing subjects according to their oxidative status and the difference was tested with the log-rank test.

All statistical analyses were performed with SPSS v.12.0 for Windows (SPSS Inc., Chicago, IL). Statistical tests were two-tailed and significant at 0.05 level.

Follow-up protocol

All interviews and specimen collections took place between October 1999 and July 2000. The personal interview was considered as the date of entry in the study. From then on, each year the institutions where the elderly lived in were contacted in order to register the date of every death event that could have occurred. In case a participant left the center, the new address was requested; when this information was not available the case was considered as a loss to follow-up ($n = 6$), and survival time was censored at the date of the last contact with the subject. According to the information provided by the institutions, none of the subjects died of accident or committed suicide. Closing date for the present analysis was considered that of the last contact with the center (between September 2004 and March 2005).

Results

During the follow-up time, there were 31 deaths in the cohort. Table I shows baseline characteristics of the sample separately for alive and dead subjects. Those who died showed higher plasma-MDA concentrations and lower α-tocopherol and β-carotene than survivors at enrolment, while age, sex, other confounding variables and all other antioxidants evaluated were not significantly different among groups.

The results of the Cox's proportional hazards regression models for tertiles of MDA and blood antioxidants and their association with mortality are presented in Table II. An age- and sex-adjusted model is presented along with a multivariate one, which includes all potential confounders measured. Except for p-GPx, which only reached statistical significance after multivariate adjustment, both models yielded similar results. We have found that e-SOD, serum α-tocopherol and β-carotene had a protective effect as their highest concentrations were associated with a 68–82% decreased chance of death. On the other hand, p-GPx was positively associated with death risk. Plasma-MDA showed a graded and significant association with mortality, in the sense that subjects in the second and third tertiles of MDA distribution had, respectively, a 3.7- and 4.2-fold increased risk of death as compared to those in the lowest one (p for trend in the multivariate model = 0.027).

A Cox's regression model was built introducing at the same time all the variables considered in Table II (including covariates) in the statistical software (Table III), showing that only plasma-MDA and serum α-tocopherol remained independently associated with mortality in the fully adjusted model. Age and self-perception of health were also confounding factors that showed an independent and significant association with mortality in the model (data not shown).

Considering the independent effect found for MDA and α-tocopherol on survival, those with the most favorable profile (low MDA and high α-tocopherol, simultaneously) were compared with those with the less favorable one (high MDA and low α-tocopherol). Characteristics of each group are shown in Table IV. Of the 31 death events in the study, 14 had high MDA and low α-tocopherol while only three cases belonged to the low MDA-high α-tocopherol group. The Kaplan–Meier curves of Figure 1 shows that subjects with high MDA and low α-tocopherol had a significantly lower probability of survival than those

Table I. Characteristics of the study sample.

	Alive (n=123)	Dead (n=31)	All (n=154)
Age (years)	72.7 ± 6.4	75.0 ± 4.4	73.2 ± 6.1
Female sex (%) (n)	59.3 (73)	45.2 (14)	56.5 (87)
Body mass index (kg/m^2)	28.4 ± 5.0	27.5 ± 4.9	28.2 ± 5.0
Daily physical activity (%) (n)	59.3 (73)	54.8 (17)	58.4 (90)
min/day⋆	76.4 ± 59.6	60.7 ± 48.5	73.4 ± 57.8
Ethanol consumption (%) (n)	27.6 (34)	22.6 (7)	26.6 (41)
g/day†	17.1 ± 16.0	27.1 ± 27.7	18.8 ± 18.5
Self-perceived health (%) (n)			
Bad/poor	35.0 (43)	54.8 (17)	39.0 (60)
Fair	13.8 (17)	6.5 (2)	12.3 (19)
Good/Excellent	51.2 (63)	38.7 (12)	48.7 (75)
Diabetes (%) (n)	16.3 (20)	12.9 (4)	15.6 (24)
Antihypertensive medication (%) (n)	22.8 (28)	29.0 (9)	24.0 (37)
Plasma albumin (g/l)	39.5 ± 3.3	39.3 ± 4.2	39.5 ± 3.5
Erythrocyte-GPx (U/g Hb)	3.5 ± 1.7	3.3 ± 1.3	3.5 ± 1.6
Plasma-GPx (U/l)	112.7 ± 100.1	130.7 ± 92.1	116.3 ± 98.5
Erythrocyte-SOD (kU/g Hb)	24.8 ± 8.1	21.8 ± 7.4	24.2 ± 8.1
Plasma-SOD (kU/l)	33.9 ± 10.4	34.5 ± 9.1	34.0 ± 10.2
Serum α-tocopherol (μmol/l)‡	21.8 ± 9.0	16.2 ± 8.5§	20.6 ± 9.2
Serum β-carotene (nmol/l)‡	184.1 ± 150.7	102.0 ± 83.2§	167.6 ± 143.4
Serum lycopene (nmol/l)‡	180.8 ± 144.8	165.6 ± 134.1	177.8 ± 142.4
Serum retinol (μmol/l)‡	1.2 ± 0.4	1.1 ± 0.5	1.2 ± 0.4
Plasma-MDA (μmol/l)	1.8 ± 1.4	2.5 ± 1.7¶	2.0 ± 1.5

Results are presented as mean ± standard deviation or as percentage.
⋆ Mean values for subjects who practised daily exercise.
† Mean values for alcohol drinkers.
‡ Adjusted for plasma lipids.
¶ Significantly different from alive subjects at $p < 0.05$ level.
§ Significantly different from alive subjects at $p < 0.01$ level.

with the inverse profile throughout the entire follow-up period ($p = 0.002$).

Discussion

The main finding of this longitudinal study is the association between oxidative stress parameters and mortality in elderly people. As far as we know, it represents the first epidemiological study which analyses the long-term effect of oxidative biomarkers along with antioxidant defenses, on survival.

SOD neutralizes superoxide radicals to form hydrogen peroxide, which GPx then reduces to yield water [18,19]. These antioxidant enzymes help prevent damage from oxygen free radicals originated in the mitochondria, to protein, lipids or DNA, by altering their structural properties and function. Lipid-soluble antioxidants, such as vitamin E or carotenoids, exert their antioxidant actions in biological membranes by breaking the chain of lipoperoxidation [19]. MDA is a major product of free radical attack to membrane polyunsaturated fatty acids and it is, probably, the most widely used biomarker of lipid peroxidation [20].

We have found that plasma-MDA concentrations predict overall mortality in elderly subjects, independently of other confounding factors. There have been reported higher plasma-MDA concentrations in older subjects [21], and this agrees with other evidence suggesting that oxidative stress increases with age [7]; but these data were correlative and only indirectly supported the association of oxidative stress with aging and death. Literature linking oxidative stress biomarkers and mortality is scarce and most of it comes from patients undergoing severe pathological conditions, in whom lipoperoxidation has been found to predict cardiovascular outcomes [22,23]. Apart from that we have only found one epidemiological study analyzing the longitudinal long-term association of MDA concentrations with risk of death in elderly subjects [24]. That study showed that higher MDA concentrations were not a risk factor for increased vascular or non-vascular mortality; but the high mean MDA in their sample (estimated as 9.4 μmol/l) raise concern that they may lack variability enough as to detect an increase in death risk of cases, since even survivors showed very high MDA concentrations. Yet, more research is urged in order to determine the prognostic significance of this biomarker in larger populations.

At this point, it should be noted a constraint regarding the specificity of oxidative stress biomarkers in relation to ageing or disease [25]. The present view of the ageing process tries to differentiate between those changes which are time-related and endogenous (senescence) and those which are disease-related [2,26]. However, most oxidative stress indices, such as MDA, cannot discriminate between the

Table II. Relative risks (RR) derived from Cox's regression survival models for comparison of tertiles of antioxidant enzymes, lipid-soluble plasma antioxidants and MDA with all-cause mortality.

		Age- and sex-adjusted RR (95% CI)		Multivariate‡ RR (95% CI)	
Erythrocyte-GPx (U/g Hb)	<2.60	1		1	
	2.61–3.89	0.94 (0.40–2.26)		1.11 (0.43–2.85)	
	>3.90	0.83 (0.35–2.00)	0.916¶	0.84 (0.42–2.89)	0.973¶
Plasma-GPx (U/l)	<55.50	1		1	
	55.51–108.00	2.32 (0.88–6.12)		3.04 (1.02–9.05)⋆	
	>108.10	2.50 (0.94–6.69)	0.154	3.54 (1.18–10.61)⋆	0.067
Erythrocyte-SOD (kU/g Hb)	<20.71	1		1	
	20.72–26.30	0.92 (0.42–1.98)		0.97 (0.43–2.23)	
	>26.31	0.32 (0.12–0.89)⋆	0.080	0.32 (0.11–0.91)⋆	0.079
Plasma-SOD (kU/l)	<29.30	1		1	
	29.31–35.90	1.99 (0.83–4.74)		1.98 (0.81–4.85)	
	>35.91	1.00 (0.40–2.53)	0.189	1.03 (0.39–2.75)	0.228
Serum α-tocopherol (μmol/l)§	<16.35	1		1	
	16.36–24.30	0.49 (0.22–1.10)		0.52 (0.23–1.20)	
	>24.31	0.20 (0.07–0.60)†	0.009	0.18 (0.06–0.56)†	0.010
Serum β-carotene (nmol/l)§	<87.00	1		1	
	87.01–177.50	0.54 (0.24–1.20)		0.47 (0.20–1.10)	
	>177.51	0.23 (0.08–0.65)†	0.016	0.22 (0.07–0.64)†	0.015
Serum lycopene (nmol/l)§	<98.00	1		1	
	98.01–205.50	0.57 (0.24–1.41)		0.65 (0.26–1.65)	
	>205.51	0.76 (0.33–1.72)	0.473	0.70 (0.29–1.73)	0.614
Serum retinol (μmol/l)§	<1.00	1		1	
	1.01–1.35	0.45 (0.19–1.06)		0.51 (0.21–1.24)	
	>1.36	0.44 (0.19–1.04)	0.079	0.48 (0.19–1.21)	0.186
Plasma-MDA (μmol/l)	<0.94	1		1	
	0.95–2.63	2.64 (0.98–7.09)		3.67 (1.27–10.58)⋆	
	>2.64	3.17 (1.19–8.42)⋆	0.061	4.22 (1.37–12.98)⋆	0.027

$n = 154$.
⋆$p < 0.05$.
†$p < 0.01$.
‡ Covariates in multivariate survival analyses: Sex, age, BMI, self-perceived health, alcohol consumption, practice of daily exercise, diabetes, use of antihypertensive drugs and plasma albumin concentration.
¶ *P*-value of test for linear trend across tertiles.
§ Adjusted for plasma lipids.

endogenously- and exogenously-induced free radical production. Although our study excluded people with diagnosed cardiovascular disease, cancer or dementia, we cannot discard that the measured MDA might be, in part, a consequence of pathological processes, as it remains unknown whether oxidative stress is an independent risk factor for disease or actually induced by other risk factors [27].

Another interesting finding in our study was the preventive effect on mortality of e-SOD, serum α-tocopherol and serum β-carotene. On the other hand, e-GPx, p-SOD, serum lycopene and retinol did not protect against all-cause risk of death, while plasma-GPx showed a direct association with mortality. e-SOD had been shown to correlate inversely with MDA [20] and, thus, its association with survival was expected taking into account the link between MDA and risk of death. e-GPx did not predict survival in the analyses, and the positive relationship found between higher GPx activity in plasma and mortality may probably reflect an adaptive response of this enzyme to increased plasma levels of lipoperoxides [28].

While physiologic oxidative stress in relation to ageing has received little attention in epidemiological surveys, dietary antioxidants have been intensively studied because of their therapeutic potential [8–13,29,30]. In most observational studies α-tocopherol has failed to predict mortality [24,31,32], while the evidence for carotenoids, in general, and β-carotene, in particular, although not conclusive [32–34], suggests that β-carotene is associated with a reduced risk of death from all causes [33]. Nevertheless, results from large-scale clinical trials have shown that supplementation with antioxidants, principally vitamin E and β-carotene, is ineffective in preventing the risk of several types of cancer or cardiovascular events, or in reducing the chance of dying by any cause. Our results show a strong and significant inverse association between α-tocopherol and β-carotene with mortality. Elderly from our sample show lower blood concentrations of these antioxidants than the reported by other studies [9,11], and this may help explain the discrepancy with our results, since it is conceivable that the role of these

Table III. Relative risks (RR) derived from the fully adjusted Cox's regression model for comparison of tertiles of antioxidant enzymes, lipid-soluble plasma antioxidants and MDA with all-cause mortality.

		RR (95% CI)†	
Erythrocyte-GPx (U/g Hb)	<2.60	1	
	2.61–3.89	1.00 (0.33–2.99)	
	>3.90	1.46 (0.49–4.37)	0.733‡
Plasma-GPx (U/l)	<55.50	1	
	55.51–108.00	1.99 (0.60–6.63)	
	>108.10	3.01 (0.87–10.37)	0.219
Erythrocyte-SOD (kU/g Hb)	<20.71	1	
	20.72–26.30	0.83 (0.29–2.35)	
	>26.31	0.39 (0.12–1.32)	0.311
Plasma-SOD (kU/l)	<29.30	1	
	29.31–35.90	1.04 (0.38–2.83)	
	>35.91	0.84 (0.27–2.61)	0.929
Serum α-tocopherol (μmol/l)¶	<16.35	1	
	16.36–24.30	0.58 (0.21–1.66)	
	>24.31	0.15 (0.03–0.71)⋆	0.058
Serum β-carotene (nmol/l)¶	<87.00	1	
	87.01–177.50	0.45 (0.16–1.29)	
	>177.51	0.41 (0.10–1.67)	0.262
Serum lycopene (nmol/l)¶	<98.00	1	
	98.01–205.50	0.64 (0.21–1.95)	
	>205.51	0.97 (0.32–3.00)	0.674
Serum retinol (μmol/l)¶	<1.00	1	
	1.01–1.35	1.11 (0.37–3.33)	
	>1.36	2.28 (0.60–8.67)	0.412
Plasma-MDA (μmol/l)	<0.94	1	
	0.95–2.63	4.93 (1.26–19.36)⋆	
	>2.64	3.04 (0.79–11.63)	0.073

$n = 154$.
⋆$p < 0.05$.
† Variables in the model: e-GPx, p-GPx, e-SOD, p-SOD, serum α-tocopherol, serum β-carotene, serum lycopene, serum retinol, plasma-MDA, sex, age, BMI, self-perceived health, alcohol consumption, practice of daily exercise, diabetes, use of antihypertensive drugs and plasma albumin concentration.
‡ *P*-value of test for linear trend across tertiles.
¶ Adjusted for plasma lipids.

antioxidants in preventing risk of disease and death becomes more relevant at these low concentrations, when a small difference in antioxidant levels could make a qualitative difference in health outcome. Besides, these studies did not evaluate the degree of oxidative stress undergone by the subjects and, so, the actual impact of their supplementation on oxidative status is unknown.

Concerning dietary antioxidants, in a previous study we had found that serum β-carotene and α-tocopherol were inversely associated with MDA in elderly subjects [20]. In the present analysis, only α-tocopherol (in addition to MDA) showed an independent effect on survival time, while β-carotene did not. It has been reported that β-carotene and α-tocopherol correlate and interact with each other [20,35] and Truscott has proposed a model of interaction, in which the antioxidant properties of β-carotene would depend on vitamin E [36]. The results of our analyses support this model and

Table IV. Characteristics of subjects with low MDA and high tocopherol as compared to those with high MDA and low tocopherol.

	Low MDA-high tocopherol ($n = 39$)	High MDA-low tocopherol ($n = 39$)
Age (years)	74.74 ± 5.74	72.36 ± 7.10
Female sex (%) (*n*)	69.2% (27)	46.2% (18)⋆
Body Mass Index (kg/m^2)	29.58 ± 4.95	27.69 ± 4.84
Plasma MDA (μmol/l)	0.64 ± 0.39	3.37 ± 1.06†
Serum α-tocopherol (μmol/l)‡	27.84 ± 6.89	14.11 ± 4.80†
Death events (%) (*n*)	7.7% (3)	35.9% (14)†

Values are mean ± standard deviation or percentage.
⋆$p < 0.05$.
†$p < 0.01$.
‡ Adjusted for plasma lipids.

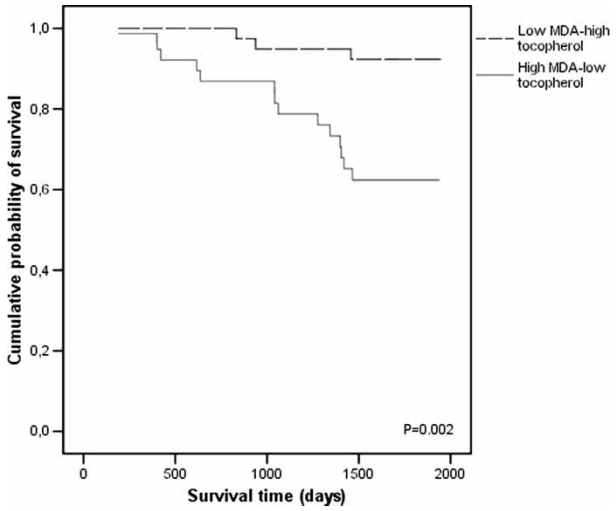

Figure 1. Kaplan–Meier survival curves for subjects with high MDA and low α-tocopherol ($n = 39$) as compared with those with low MDA and high α-tocopherol ($n = 39$).

reveal α-tocopherol as the antioxidant molecule most directly associated with survival, among those considered by us. On the other hand, the independent association between α-tocopherol and survival, regardless of MDA, suggests that prevention of lipoperoxidation would not be the only pathway by which this vitamin would exert its protective activity. In this sense, recent research have characterized multiple non-antioxidant actions of α-tocopherol, including transcriptional modulation of gene expression, inhibition of cell proliferation, platelet aggregation and monocyte adhesion or activation/inhibition of several enzymatic activities [37,38].

When considering the oxidant/antioxidant balance regarding α-tocopherol and MDA levels simultaneously, we found only three death events (8%) in the low MDA-high tocopherol group as opposed to near 36% of cases among those with high MDA and low tocopherol, which points out the importance of maintaining adequate levels of both factors. Also, it is interesting to note the unequal sex ratio between groups, with women being predominant in the low mortality group and men in the high mortality one, a consequence of the higher percentage of women in the high-tocopherol group (data not shown).

Some limitations should be considered in this study. The first one refers to the specificity of MDA as a marker of oxidative stress; as discussed above, it cannot discriminate between endogenously- and environmentally-induced lipid peroxidation and this concerns its relevance in the context of the free radical theory of ageing, although, it does not modify the potential utility of MDA for predicting risk of mortality. Secondly, the sample size is not very large and, so, prolonging the follow-up period for some more years will obviously yield more solid results, although the coherence, stability and strength of the associations found allow us to feel confident about the findings.

In conclusion, results of the present study support the free radical theory of ageing since we have found that risk of death is associated with a higher level of lipid peroxidation and lower levels of antioxidant defense mechanisms such as e-SOD, α-tocopherol and β-carotene. The potential utility of plasma-MDA for predicting risk of mortality in the elderly needs further investigation.

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