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Oxidative stress status in an institutionalised elderly group after the intake of a phenolic-rich dessert

M. Carmen Ramirez-Tortosa[1]*, Javier García-Alonso[2], M. Luisa Vidal-Guevara[3], José L. Quiles[4], M. Jesús Periago[2], Javier Linde[1], M. Dolores Mesa[1], Gaspar Ros[2], Pedro Abellán[3] and Ángel Gil[1]

[1]*Department of Biochemistry and Molecular Biology, Institute of Nutrition and Food Technology, University of Granada, Campus de Cartuja, 18071 Granada, Spain*
[2]*Department of Food Science and Nutrition, Veterinary Faculty, University of Murcia, Murcia, Spain*
[3]*Department of Research and Development, Hero España S.A., Alcantarilla, Murcia, Spain*
[4]*Department of Physiology, Institute of Nutrition and Food Technology, University of Granada, Campus de Cartuja, 18071 Granada, Spain*

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The elderly population undergoes a series of physiological and sociological changes common to old age with a high probability of suffering degenerative illness and malnutrition. A dessert rich in phenolic compounds has been designed by using concentrated juices of grape, cherry, blackberry, blackcurrant and raspberry with the aim of it being used as a complementary food in adulthood. In the present study, we investigated the effect of the intake of this dessert (a jar of 200 g daily for a period of 2 weeks), with an antioxidant activity equivalent to ten servings of fruits and vegetables, on several markers of oxidative and antioxidant status in DNA and plasma in a group of elderly individuals. Non-smoking institutionalised elderly subjects were recruited from a pool of volunteers in an old-age home in Murcia (Spain). Twenty-two subjects (six men and sixteen women) participated in the study. The study was designed as a randomised intervention trial with a period of 2 weeks. At days 1 and 15, blood samples were collected to analyse total antioxidant capacity, biochemical parameters, antioxidant vitamins, LDL peroxidation, and DNA damage in peripheral blood lymphocytes. The conclusion of the present study is that a 2-week intervention with our dessert enriched with natural polyphenol compounds in elderly individuals does not give enough time to find changes in the antioxidant and oxidative status. Also, the view that the marked antioxidant ability of polyphenols *in vitro* does not translate to analogous effects *in vivo* was confirmed. Moreover, a highly oxidative stress status during ageing was confirmed, together with the need to perform follow-up nutritional studies to improve this situation.

Phenolic-rich dessert: Ageing: Antioxidant status: DNA damage

The antioxidant properties of foods in relation to health and particularly on the maintenance and protection from degenerative diseases are of growing interest among scientists, food manufacturers, consumers and health organisations. Since the 1990s, several international organisations have recommended increasing the consumption of fruits and vegetables to five or more daily servings, in order to provide a desirable intake of antioxidants and to improve human health (World Health Organization, 1990; World Cancer Research Foundation & American Institute for Cancer Research, 1997).

However, for certain groups of the population, such as the elderly, it is difficult to consume those daily amounts of fruits and vegetables. The elderly population undergoes a series of physiological and sociological changes common to old age with a high probability of suffering degenerative illness and malnutrition (Tucker & Buranapin, 2001). During the ageing process, a more sedentary lifestyle, resulting in less energy expenditure, poor appetite, dental disease, alterations in absorption and metabolism of several nutrients, medication, etc. may lead to a decline in the intake of macro- and micronutrients. Consequently, elderly individuals are at risk of a sub-optimal nutritional state or multiple micronutrient deficiencies (De Jong, 1999). In a recent study carried out with 10 208 participants from eight random population studies and participants in fifty-seven

Abbreviations: ABTS, 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate); FRAP, ferric-reducing ability of plasma; TBARS, thiobarbituric-acid-reactive substances; TEAC, Trolox-equivalent antioxidant capacity.

* **Corresponding author:** Dr M. Carmen Ramirez-Tortosa, fax +34 958248326, email mramirez@ugr.es

studies included in a meta-analysis, it has been shown that the vitamin status of the Spanish population clearly shows room for improvement, especially with regard to vitamins A, E, D, B_2, B_6, and folates (Ortega *et al.* 2003). For this reason, there is a trend in the food industry towards functional foods with healthy effects based, among others, on their antioxidant properties (Karakaya *et al.* 2001).

Antioxidant vitamins, including vitamin C, vitamin E and a variety of phytochemicals, are important in maintaining effective antioxidant defences against oxidant stress-related diseases, including cancer, cataracts and Alzheimer's disease. Berries, grapes and cherries are recognised as fruits with a high content of antioxidants. The antioxidant properties of these fruits are believed to be due to the content of anthocyanins and other phenolic compounds. Similarly, concentrated juices with a high content of phenolics should probably exhibit an effect (García-Alonso *et al.* 2002). In this basis, a dessert has been designed by using concentrated juices of grape, cherry, blackberry, blackcurrant and raspberry with the aim of it being used as a complementary food in adulthood. The antioxidant activity of this product is considered similar to that of red wine and higher than that of many fruits and vegetables. In previous studies, we have observed that the antioxidant capacity of this dessert during storage remained practically invariable for 1 year at different temperatures (8, 21 and 30°C; García-Alonso *et al.* 2003). In the present study, we investigated the effect of the intake of this dessert (a jar of 200 g daily for a period of 2 weeks), with an antioxidant activity equivalent to ten servings of fruits and vegetables, on several markers of oxidative and antioxidant status in DNA and plasma in a group of elderly individuals.

Subjects and methods

Test product

The test product was an experimental dessert prepared by the Department of Research and Development of Hero Spain S.A. (Alcantarilla, Murcia, Spain). This product was formulated and designed using the data of total antioxidant activity available in the scientific literature for the fruits used (Cao *et al.* 1996; Wang *et al.* 1996). The objective was to reach, per serving (a jar of 200 g), an average antioxidant capacity equivalent to ten servings of fruits and vegetables, 2-fold higher than the 'five a day' recommended by several international organisations (World Health Organization, 1990; World Cancer Research Foundation and American Institute for Cancer Research, 1997).

The major ingredient was water, which was mixed with commercially available concentrated juices of grape (26 %), cherry (2 %), blackberry (0·6 %), blackcurrant (0·6 %) and raspberry (1 %). Pectin was added to jellify the product. The resulting product was pasteurised in order to obtain a microbiologically stable foodstuff and the product was bottled hot in jars to ensure headspace vacuum.

For the characterisation of the dessert, several physical–chemical parameters and nutritional composition were analysed. Total titratable acidity, pH and soluble solids were measured following the procedures described by Shams & Thompson (1987). Proximate composition of the dessert (moisture, ash, total protein, total fat and total dietary fibre) was analysed by the official methods of the AOAC International (1999) and energy was calculated based on the macronutrient composition. Total phenols in the dessert were analysed spectrophotometrically using a Folin–Denis reagent following the AOAC International (1999) method. The major phenolic compounds were analysed by HPLC according to the method described by Cantos *et al.* (2000) and vitamin C content was measured by HPLC, as described by Esteve *et al.* (1995). The *in vitro* total antioxidant activity was assessed by the Trolox-equivalent antioxidant capacity (TEAC) assay and by the ferric-reducing ability of plasma (FRAP) assay, as described later (p. 945). All analyses were made in 3of proofs and the results are shown in Table 1.

Study design

Non-smoking institutionalised elderly subjects were recruited from a pool of volunteers in an old-age home in Murcia (Spain). The protocol was carefully explained to the volunteers and their written informed consent was

Table 1. Average daily intake of energy and nutrients in a group of elderly individuals*

(Mean values and standard deviations)

	RDA†		Average daily intake (*n* 17)	
Nutrient	Men	Women	Mean	SD
Energy (kJ)	9614	7942	7610	1009
Energy (kcal)	2300	1900	1821	242
Proteins (g)	63	50	76	13
Carbohydrates (g)	–	–	213	37
Lipids (g)	–	–	76	14
Saturated fatty acids (g)	–	–	19	4
MUFA (g)	–	–	29	8
PUFA (g)	–	–	10	4
Cholesterol (mg)	<310	<310	264	50
Dietary fibre (g)	25	25	18	5
Vitamin A (μg)	1000	800	1526	1183
Vitamin B_1 (mg)	1·2	1	1·4	1·1
Vitamin B_2 (mg)	1·4	1·2	1·57	0·61
Vitamin B_6 (mg)	2	1·6	1·8	0·2
Vitamin B_{12} (μg)	2	2	5·3	2·8
Vitamin C (mg)	60	60	157·6	85
Vitamin D (μg)	5	5	3·5	2·2
Vitamin E (mg)	10	8	5·7	1·6
Niacin (mg)	15	13	20·1	3·9
Folic acid (μg)	200	180	248·3	76·1
Na (mg)	–	–	1443	342
K (mg)	–	–	2955	524
Ca (mg)	800	800	859	151
P (mg)	800	800	1144	168
Ca:P ratio	1	1	0·76	0·14
Mg (mg)	350	280	272·5	51·6
Fe (mg)	10	10	12·4	2·5
Zn (mg)	15	12	6·3	1·5
I (μg)	150	150	59·3	17·1

* For details of subjects and procedures, see p. 944.
† According to the National Research Council (1989) for men and women over 50 years old.

obtained. Twenty-two subjects (six men and sixteen women) participated in the study. The average age was 78·88 (range 65–92) years and average BMI was 30·98 (range 23·83–37·58) kg/m^2. A control group of elderly volunteers (n 8) who did not receive the treatment was included in order to establish that any changes in parameters were not merely due to the effects of time. A non-smoking, healthy group of twelve subjects (six men and six women; age range 25–50 years), recruited from a pool of volunteers of Hero Spain S.A. (Alcantarilla, Murcia, Spain), were also studied to serve as a healthy reference group.

The present study was approved by the local research ethics committee of Murcia University and by the local government (ISSORM, Murcia, Spain) and complied with Helsinki guidelines for clinical studies.

The present study was designed as a randomised intervention trial with a period of 2 weeks. The subjects received the dessert and were instructed to maintain their usual diet and to consume one serving (a jar of 200 g) of test product daily for 2 weeks, at a self-selected time but not replacing a meal. The subjects were also instructed to store the dessert in a refrigerator. The habitual diet of the subjects was checked daily with 24 h dietary recalls and the content of macronutrients and selected micronutrients in the diet was calculated using the computer program ALIMENTACIÓN Y SALUD 0698.046 (BitASDE General Médica Farmacéutica, Valencia, Spain). At days 1 and 15, blood samples were collected to analyse total antioxidant capacity, biochemical parameters, antioxidant vitamins, LDL peroxidation, and DNA damage to peripheral blood lymphocytes. Five women withdrew during the study due to their dislike of the test product and did not finish all the experiments. Data for twenty-two subjects were available on day 1 (elderly baseline group) and data for seventeen subjects were available at the end of the study (elderly treated group). To compare the results of the analysed parameters, blood samples were also collected from the non-smoking, healthy reference group.

Blood sample collection

At baseline and at the end of the study period, blood samples were collected by venepuncture from fasting subjects. For the analysis of total antioxidant capacity, ascorbic acid, Fe, albumin, bilirubin and uric acid in serum, 10 ml blood were collected into evacuated glass tubes (Venoject; Terumo, Leuven, Belgium) and allowed to clot at room temperature for 25 min. Samples were immediately centrifuged at 1000 ***g*** for 15 min at 4°C to recover the serum. Serum samples were deproteinised for ascorbate determination before freezing and stored at −80°C until analysed. For analysis of retinol, α-tocopherol, β-carotene and ubiquinol in plasma, lipid peroxidation markers and DNA damage to peripheral blood lymphocytes, 10 ml blood were collected into evacuated glass tubes containing K_3–EDTA (Venoject). Samples were refrigerated and transported within 4 h to the Institute of Nutrition and Food Technology (Granada, Spain).

Serum total antioxidant capacity

In order to overcome problems and eliminate the tedious determination of individual antioxidants, methods capable of measuring the antioxidant activity of all the compounds present in a sample with one simple determination have been developed, including the TEAC and the FRAP assays. These total antioxidant assays are useful in getting a global picture of relative antioxidant activities in foods, body fluids and tissues, and how they change in clinical, physiological and pathological conditions.

Serum TEAC was measured by using the method of Miller *et al.* (1993) with commercially available kits (Total Antioxidant Status, NX 2332; Randox Laboratories Ltd, Crumlin, Co. Antrim, UK). This method is based on the inhibition by antioxidants of the absorbance of the radical cations of 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS) at 600 nm. ABTS radical cations are formed by the incubation of ABTS with metmyoglobin and H_2O_2. The final results are expressed as mmol Trolox equivalents/l. The inhibition of 1 Trolox equivalent/l equals the inhibition produced by 1 mmol Trolox/l. Serum FRAP was determined by the method of Benzie & Strain (1996). The FRAP assay measures the ferric-reducing ability of plasma or serum. At low pH, when a ferric (Fe^{3+})–tripyridyltriazine complex is reduced by antioxidants to the ferrous (Fe^{2+}) form, an intense blue colour with an absorption maximum at 593 nm develops. In the FRAP assay, Fe^{2+} was used as a standard. The final results were expressed as mmol Fe^{2+} equivalents/l. The unit of 1 Fe^{2+} equivalent/l equals the amount of Fe^{2+}/l required to give the same absorbance change.

Biochemical parameters

Fe, albumin, bilirubin and uric acid were measured in serum by using a Cobas Mira Plus Chemistry Analyser (ABX Diagnostics, Montpellier, France) with reagent kits purchased from ABX Diagnostics.

Co-enzyme Q_{10} and antioxidant vitamin determination

For ascorbic acid (vitamin C) determination, serum samples were added to one volume of 10 % (w/v) metaphosphoric acid containing 0·54 mmol Na_2–EDTA, agitated in a vortex mixer and centrifuged at 7200 ***g*** for 5 min to pellet the precipitated proteins. The supernatant fraction was removed and stored at −80°C until analysed. Serum ascorbic acid was assayed by using reagent kits for colorimetric determinations (Böehringer-Mannheim, 409677, Mannheim, Germany).

Analyses of co-enzyme Q_{10}, retinol, β-carotene and α-tocopherol were assayed according to MacCrehan (1990) by reversed-phase HPLC using a Spherisorb S5 ODS1 (Merck, Darmstadt, Germany) column and ethanol–purified water (97:3, v/v) as the mobile phase. The HPLC system was a Beckman in-line diode array detector; model 168 (Fullerton, CA, USA) connected to a Waters 717 plus autosampler (Milford, MA, USA). The column was maintained at a constant temperature of 22°C. Co-enzyme Q_{10}, retinol, β-carotene and α-tocopherol were

identified by predetermining the retention times of individual pure standards.

Determination of low-density lipoprotein oxidation susceptibility

LDL was isolated as described by Chung *et al.* (1981). LDL protein was measured by the Bradford (1979) method. To study the susceptibility to oxidation of LDL, two determinations were performed; thiobarbituric-acid-reactive substances (TBARS) and conjugated dienes. LDL protein (100 mg/l) was oxidised in the presence of Cu^{2+} (10 and 20 μmol/l) in PBS for 6 h at 37°C (Jialal & Grundy, 1991). The lipid peroxide content of oxidised LDL was determined as TBARS according to Buege & Aust (1978). Conjugated dienes in LDL were carried out according to Puhl *et al.* (1994) in a Perkin Elmer UV-VIS Lambda 40 spectrometer (Fremont, CA, USA) equipped with an auto-cell holder and controlled by a Peltier element at the temperature of 37°C. The lag phase and slope were calculated using the Perkin Elmer UV-WINLAB software.

DNA oxidative damage (comet assay)

Peripheral blood was collected and the 'buffy coat', enriched in erythrocytes, was removed, diluted 1:1 with RPMI-1640 medium, layered onto an equivalent volume of Histopaque to obtain peripheral blood lymphocytes. The comet assay was used to measure DNA strand breaks in the cells (Collins *et al.* 1996).

Statistical analyses

Before any statistical analysis, all variables were checked for normality and homogeneous variance using the Kolmogorov–Smirnoff and the Levene tests, respectively. When a variable was found not to follow normality, it was log-transformed and reanalysed. A Student's *t* test was performed to evaluate differences between baseline and after-treatment parameters in the elderly individuals. All parameters for the elderly baseline, elderly treated and healthy reference groups were analysed by a one-way ANOVA; to evaluate mean differences from elderly baseline and elderly treated groups *v.* healthy reference group a multiple comparison test adjusted by Bonferroni corrections was performed. A P value of less than 0·05 was considered significant. Data were analysed using a statistical software package (SPSS for Windows, 11.0.1.; SPSS Inc., Chicago, IL, USA).

Results

Compositional indices, total antioxidant capacity, phenolic profile and vitamin C content in the dessert made of grapes, cherries and berries are shown in Table 2. The product showed a high water content, whilst the concentrations of the nutrients such as protein, fibre and minerals were very low, with levels under 0·5 % of total weight. Fat was not detected in the compositional analysis. Total phenol content was 1904·21 mg/kg, whereas the main phenolics were anthocyanins, followed by hydroxycinnamic acids, stilbenoids, flavonols and ellagic acids. This product showed a high antioxidant capacity with a mean activity of 18·22 mmol TEAC/l and 23·65 mmol Fe^{2+}/l, for both methods assayed.

Table 2. Compositional indices, total antioxidant activity, phenolics profile and vitamin C content of the dessert*

(Mean values and standard deviations)

	Mean	SD
Energy (kJ/100 g)†	338·00	2·10
Energy (kcal/100 g)†	81·00	0·50
Moisture (%)	79·18	0·10
Total protein (%)	0·50	0·02
Total fat (%)	nd	
Total dietary fibre (%)	0·28	0·00
Ash (%)	0·34	0·02
Total soluble solids (°Brix)	21·00	1·12
pH	3·65	0·01
Total titratable acidity (% citric acid)	0·62	0·02
TEAC (mmol/l)	18·22	1·35
FRAP (mmol/l)	23·65	0·85
Total phenols (mg/kg)	1904·21	89·58
Anthocyanins (mg/kg)	224·50	1·10
Hydroxycinnamic acids (mg/kg)	71·97	2·97
Stilbenoids (mg/kg)	4·70	0·45
Flavonols (mg/kg)	30·73	1·40
Ellagic acids (mg/kg)	3·24	0·14
Vitamin C (mg/kg)	138·80	0·50

nd, Not detected; °Brix, beverage such as juice; TEAC, Trolox-equivalent antioxidant capacity; FRAP, ferric-reducing ability of plasma.
* For details of procedures, see p. 945.
† Calculated based on the macronutrient composition.

The average daily intake of energy and nutrients (Table 1) was compared with the RDA according to the National Research Council (1989). Total energy intakes for women and men were below the RDA, but the subjects showed a stable weight (body-weight changes were less than 1 kg) during the intervention period. As regards the macronutrients, there were high intakes of protein and fat, which represented 16 and 36 % of the total energy intake, respectively. The intake of micronutrients showed a high variability, depending on the subjects. Vitamin A and vitamin C intakes met the RDA but showed a large standard deviation. The diet provided only vitamins D and E in amounts below the RDA, showing the same pattern as the total intake of Zn and I.

No statistical differences were found in all parameters analysed between elderly subjects after the intake of the high antioxidant dessert and the elderly control group at the end of the study.

Serum antioxidant capacity and biochemical measures obtained from the subjects after treatment compared with the baseline and the healthy reference group are shown in Table 3. There were no significant differences in the antioxidant activity and the biochemical parameters measured in the serum of elderly individuals after the intervention study period. In addition, the data of TEAC and FRAP of the elderly individuals were not different than those obtained in the healthy reference group. Only uric acid was significantly lower ($P<0·05$) in the healthy reference group compared with data from the elderly individuals.

Table 3. Serum total antioxidant capacity and biochemical measures in elderly volunteers consuming a phenolic-rich dessert* (Mean values and standard deviations)

Variable	EB (*n* 22)		ET (*n* 17)		EC (*n* 8)		HR (*n* 12)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TEAC (mmol/l)	0·62[a]	0·14	0·66[a]	0·16	0·58[a]	0·21	0·65[a]	0·14
FRAP (mmol/)	0·96[a]	0·16	1·01[a]	0·17	0·82[a]	0·09	0·95[a]	0·19
Albumin (g/l)	39·4[a]	5·7	40·1[a]	3·9	36·0[a]	4·4	46·2[b]	3·1
Bilirubin (μmol/l)	11·11[a]	5·59	12·73[a]	5·93	11·12[a]	5·5	10·7[a]	3·9
Fe (μmol/l)	12·9[a]	6·8	15·30[a]	6·2	13·1[a]	3·1	15·0[a]	5·9
Uric acid (μmol/l)	281[ab]	72	300[b]	77	240[a]	70	251[a]	88
Ascorbic acid (μmol/l)	51[a]	26	53[a]	15	38[a]	11	55[a]	22

EB, elderly baseline group; ET, elderly after treatment group; EC, elderly control group; HR, healthy reference group; TEAC, Trolox-equivalent antioxidant capacity; FRAP, ferric-reducing ability of plasma.
[a,b]Within a row, mean values with unlike superscript letters were significantly different ($P<0·05$).
* For details of subjects and procedures, see p. 944.

No differences were found in plasma lipid antioxidant vitamins (α-tocopherol, β-carotene and retinol) and co-enzyme Q_{10} values in elderly subjects after the intake of the high antioxidant dessert (Table 4). It is important to emphasise that plasma antioxidant vitamin levels (α-tocopherol, retinol, β-carotene) and co-enzyme Q_{10} in the elderly subjects were below those considered as normal values. (Human healthy averages are: retinol, 2·27 μmol/l; α-tocopherol, 23·5 μmol/l; β-carotene, 0·35 μmol/l; co-enzyme Q_{10}, 0·60 μmol/l; Cutler & Mattson, 2003.) However, there were significant differences for these vitamins between the elderly and healthy groups. Susceptibility of LDL to oxidation did not change with the intake of the dessert in the elderly volunteers but the LDL TBARS levels and LDL lag phase were significantly different from the healthy group, showing more LDL oxidation in the elderly subjects (Table 4).

DNA strand breakage did not decrease in the elderly institutionalised individuals after the intake of the high antioxidant dessert but their values were significantly higher compared with the healthy reference group (Fig. 1).

Discussion

Ageing is usually defined as the progressive loss of function accompanied by decreasing fertility and increasing mortality with advancing age (Kirkwood & Austad, 2000). The importance of ageing is based, first, in the high percentage of individuals over 65 years (close to 20 %) and the rise in the number of individuals over 80 years and, second, in the growing incidence of ageing-related chronic diseases such as Alzheimer's disease, Parkinson's disease, diabetes and cancer. Moreover, a leading cause of death among older individuals worldwide is vascular disease and associated chronic conditions. The impact of the diet and dietary components on ageing and age-associated degenerative diseases has been widely recognised in recent years (Ames *et al.* 1993; Meydani, 2001). Accordingly, there is great potential for the prevention of these diseases through healthy lifestyles that include physical activity and well-balanced diets (Tucker & Buranapin, 2001).

There is increasing evidence that the oxidation of biomolecules (DNA, proteins and lipids) may play a role in

Table 4. Plasma lipid antioxidant vitamins and co-enzyme Q_{10} values, and susceptibility of low-density lipoproteins to oxidation in elderly volunteers consuming a phenolic-rich dessert*
(Mean values and standard deviations)

Variable	EB (*n* 22)		ET (*n* 17)		EC (*n* 8)		HR (*n* 12)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma								
α-Tocopherol (μmol/l)	19·0[a]	6·3	16·7[a]	4·36	17·7[a]	5·4	20·5[a]	3·8
Retinol (μmol/l)	0·85[a]	0·21	0·74[a]	0·21	0·80[a]	0·30	1·5[b]	0·4
β-Carotene (μmol/l)	0·29[a]	0·1	0·29[a]	0·1	0·41[a]	0·2	0·8[b]	0·4
Co-enzyme Q_{10} (μmol/l)	0·18[a]	0·08	0·26[a]	0·15	0·15[a]	0·03	0·52[b]	0·19
LDL								
Conjugate dienes								
Phase-lag (min)	53·8[a]	12	55·1[a]	11	53·3[a]	13·3	81·3[b]	27
Slope	0·02[a]	0·09	0·02[a]	0·009	0·014[a]	0·004	0·02[a]	0·01
TBARS (nmol/mg LDL protein)								
10 μM (Cu^{2+})	27·7[b]	10·8	34·16[b]	10·5	27·4[b]	11·5	19·2[a]	4·7
20 μM (Cu^{2+})	33·9[b]	12·0	30·05[b]	11·1	30·5[b]	11	20·3[a]	3·0

EB, elderly baseline group; ET, elderly after treatment group; EC, elderly control group; HR, healthy reference group; TBARS, thiobarbituric-acid, substances.
[a,b]Within a row, mean values with unlike superscript letters were significantly different ($P<0·05$).
* For details of subjects and procedures, see p. 944.

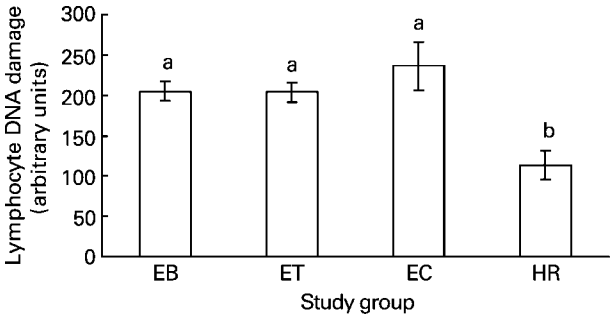

Fig. 1. Peripheral lymphocyte DNA strand breakage after the intake of a high antioxidant dessert. Values are means, with standard deviations represented by vertical bars. EB, elderly baseline group (*n* 22); ET, elderly after treatment group (*n* 17); EC, elderly control group (*n* 8); HR, healthy reference group (*n* 12). Mean values with unlike superscript letters were significantly different ($P<0{\cdot}05$).

the susceptibility to disease, especially in ageing-related conditions such as cancer and heart disease, and in the ageing process itself (Pryor, 1987; Halliwell & Chirico, 1993). Animal data have shown that DNA damage accumulates with age (Richter *et al.* 1988) and, as Harman (1956) suggested almost half a century ago in his free radical theory of ageing, oxidative damage is related to the debilities associated with ageing. Consequently, it would seem that the oxidative stress status and antioxidant status of the elderly population should be of considerable interest and importance. The present results are in agreement with those of Trevisan *et al.* (2001). These authors demonstrated that ageing is associated with increased oxidative stress and reduced antioxidant potentials, as has been reported in the present study for the low TEAC, FRAP, plasma antioxidant vitamin values and the higher susceptibility of LDL to Cu-induced oxidation in elderly volunteers at the beginning of the study compared with the healthy reference group.

In the last few years, much attention has been focused on the antioxidant properties of flavonoids, a large class of polyphenolic compounds derived from plants. Evidence suggests that these compounds may protect tissues against damage caused by oxygen free radicals and lipid peroxidation (Bub *et al.* 2003). The antioxidant capacity of the dessert used in the present study was higher than that observed in many fruits and vegetables, being similar to that of antioxidant beverages such as tea and red wine (García-Alonso *et al.* 2003). The antioxidant activity of the product seems to be related to its phenolic compounds, since there was a positive and significant correlation between anthocyanin content and total antioxidant activity as assessed by the TEAC and FRAP assays.

We thought that maybe supplementation with flavonoids in our elderly volunteers would help to improve their antioxidant status and consequently attenuate their oxidative status.

The main flavonoid used in our dessert was anthocyanin (224 mg/kg). We have previously described anthocyanin as endowed with antioxidant effects because it decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats (Ramirez-Tortosa *et al.* 2001). Furthermore, other studies have reported that anthocyanins are absorbed in elderly women, finding a high level of these compounds in plasma and urine after their intake (Cao *et al.* 2001). However, we did not find a significant effect after supplementation with our dessert on biological markers of oxidative stress, plasma antioxidant defence and LDL oxidisability in the elderly volunteers and, maybe, to get effects as antioxidants requires doses far in excess of that which is nutritionally relevant. Therefore, the present results have confirmed the view that the marked antioxidant ability of polyphenols *in vitro* does not translate to analogous effects *in vivo*.

The present results are in accordance with Young *et al.* (2002), who found that an intervention in healthy human subjects with 18·6 mg catechin/d for 6 weeks did not affect markers of oxidative stress and antioxidant status, including plasma or haemoglobin protein oxidation, plasma oxidation and plasma lipid antioxidant vitamins. Furthermore, Hininger *et al.* (2001) did not find any effect of lutein (15 mg), lycopene (15 mg) or β-carotene (15 mg) supplementation on biochemical indices of oxidative status in healthy adult males after 3 months of intervention. In all these studies the intervention period was longer than in the present research. However, it is reasonable to think that since the product tested here is much more rich in antioxidants than the amounts used in those mentioned studies, a study period of 2 weeks should be enough to find an improvement in the oxidative stress status of the subjects.

Oxidative DNA damage accumulates with age and is related to the lifespan of the particular organism, being also associated with premature ageing (Beckman & Ames, 1998). Antioxidants protect the cellular system from oxidative damage (Krinsky, 1992) and the consumption of foods rich in antioxidants such as vitamin E, vitamin C and polyphenols is associated with a decreased risk for cancer and coronary disease (Byers & Perry, 1992; Hertog *et al.* 1995). The study of biomarkers of DNA damage (for example, the comet assay) and biochemical markers (for example, plasma antioxidants) as putative indicators of ageing is used increasingly to provide a focused and mechanistic approach to the study of diet, health and disease. Using this approach, we decided to measure the potential effect of our dessert rich in flavonoids on DNA damage. Unfortunately, we did not find any effect on DNA strand breakage. The reason for this, maybe, is the short intervention period (2 weeks) and the poor antioxidant status of the elderly volunteers confirmed by the high endogenous DNA damage at the beginning of the study. However, Pool-Zobel *et al.* (1997) showed that endogenous DNA strand breakage was reduced in human lymphocytes isolated from subjects given supplemental vegetable juice (tomato juice with lycopene, carrot juice with β-carotene and spinach with lutein in water) for 2 weeks each. Other studies showed that prolonged supplementation (80 d) with a commercially available fruit and vegetable extract also decreased DNA strand breakage in elderly volunteers (Smith *et al.* 1999). Lymphocytes isolated from human volunteers fed a diet supplemented with lycopene (16·5 mg) for 21 d were more resistant to *ex vivo* H_2O_2 treatment compared with lymphocytes from the untreated controls (Riso *et al.* 1999).

It can be considered that any changes in the parameters were not due to the effect of time because no differences between elderly subjects after the intake of the high-antioxidant dessert and the elderly control group at the end of the study were found. Finally, it is important to keep in mind that elderly subjects have a risk for marginal deficiency of lipid antioxidant vitamins such as α-tocopherol, retinol and β-carotene. Dietary components with a high antioxidant activity have to receive particular attention because of their potential role in modulating oxidative stress associated with ageing and chronic conditions (Meydani, 2001).

The conclusion of the present study is that an intervention of 2 weeks with our dessert enriched with natural polyphenol compounds in elderly individuals does not provide enough time to find changes in the antioxidant and oxidative status. We have confirmed the view that the marked antioxidant ability of polyphenols *in vitro* does not translate to analogous effects *in vivo*. Moreover, a highly oxidative stress status during ageing was confirmed, together with the need to conduct follow-up nutritional studies to improve this situation.

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