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In Vitro Antioxidant Activities of Edible Artichoke (*Cynara scolymus* L.) and Effect on Biomarkers of Antioxidants in Rats

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Artichoke (*Cynara scolymus* L.), an edible vegetable from the Mediterranean area, is a good source of natural antioxidants such as vitamin C, hydroxycinnamic acids, and flavones. The antioxidant activity of aqueous–organic extracts of artichoke were determined using three methods: (a) free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) scavenging, (b) ferric-reducing antioxidant power (FRAP), and (c) inhibition of copper(II)-catalyzed in vitro human low-density lipoprotein (LDL) oxidation. In addition, the present study was performed to investigate the ability of the edible portion of artichoke to alter in vivo antioxidative defense in male rats using selected biomarkers of antioxidant status. One gram (dry matter) had a DPPH• activity and a FRAP value in vitro equivalent to those of 29.2 and 62.6 mg of vitamin C and to those of 77.9 and 159 mg of vitamin E, respectively. Artichoke extracts showed good efficiency in the inhibition in vitro of LDL oxidation. Neither ferric-reducing ability nor 2,2′-azinobis-(3-ethylbenzothiazolin-6-sulfonate) radical scavenging activity was modified in the plasma of the artichoke group with respect to the control group. Among different antioxidant enzymes measured (superoxide dismutase, gluthatione peroxidase, gluthatione reductase, and catalase) in erythrocytes, only gluthatione peroxidase activity was elevated in the artichoke group compared to the control group. 2-Aminoadipic semialdehyde, a protein oxidation biomarker, was decreased in plasma proteins and hemoglobin in the artichoke-fed group versus the control group. In conclusion, the in vitro protective activity of artichoke was confirmed in a rat model.

KEYWORDS: Artichoke; polyphenols; antioxidant activity; radical scavenging; redox potential; low-density lipoprotein oxidation; biomarkers; protein oxidation; rats

INTRODUCTION

The consumption of foods of vegetable origin has been associated with reduced risk of a range of chronic diseases (*1*, *2*). Because of their antioxidant and free radical scavenging properties, constituents such as vitamins, polyphenols, and carotenoids may play a role in the etiology of chronic diseases through modulation of oxidative damage to body cells and molecules.

Artichoke (*Cynara scolymus* L.), an edible vegetable from the Mediterranean area, is a good source of natural antioxidants such as vitamin C, hydroxycinnamic acids, and flavones. Artichoke is of considerable economic importance for Spain and other countries. The Spanish annual production of artichoke is estimated at ~273000 tons (*3*). Extracts from artichoke have been used in folk medicine against liver complaints, and such extracts have been claimed to enhance detoxification reactions in the liver. Interestingly, it has been suggested that at least some of these effects are due to the potential antioxidant activity of the polyphenolic fraction (*4*, *5*). Recently, in vitro experiments have been conducted to evaluate artichoke activities on human low-density lipoprotein (LDL) measuring malondialdehyde (MDA) products, showing that the simultaneous incubation of LDL with extracts of artichoke and cupric for 8 h reduced the oxidation of LDL induced by metal (*6*).

The antioxidant activity of vegetable foods includes several mechanisms such as radical scavenging, metal quelation, and quenching of singlet oxygen. An evaluation of the antioxidant capacity of a compound or group of compounds has to include this multifunctional activity (*7*–*10*). Thus, to evaluate in vitro this potential antioxidant activity it is necessary to assess several assays, which include a battery of different antioxidative mechanisms (*8*, *11*, *12*). On the other hand, to evaluate the potential antioxidant effect of foods from vegetable origin in biological systems it is useful to measure, along with the total

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Table 1. Composition of Test Diets Used in the Feeding Experiment[a]

	control (g or kcal per kg of diet)	exptl (g or kcal per kg of diet)
casein	140	140
cornstarch	435.5	377.3
dextrose	155	155
saccharose	100	100
cellulose	80	-
tert-butylhydroquinone	0.008	0.008
AIN-93M mineral mixture[a]	35.0	35.0
AIN-3VX vitamin mixture[a]	10.0	10.0
L-cystine	1.8	1.8
choline bitartrate	2.5	2.5
soy oil	40.0	40.0
artichoke (edible portion)		138.2
energy	3509.4	3670.3

[a] The mineral and vitamin mixture composition was prepared according to the method of Reeves et al. (*16*).

antioxidant activity of the plasma, several primary and secondary products of oxidative damage (*13*−*15*).

In the present study we have investigated in a first step in vitro antioxidative properties of extracts from artichoke by a multifunctional methodology. Second, we have evaluated the effect of the intake of artichoke during 3 weeks on several biomarkers of antioxidant status (AS) such as protein oxidation, total antioxidant activity, and antioxidative enzymes in different blood compartments of male rats.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water-soluble analogue of vitamin E, and stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) were from Aldrich Chemical Co. (St. Louis, MO). 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) was from Fluka Chemicals (Madrid, Spain). 2,2′-Azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS), NADPH, glutathione, FAD, purpald, and potassium periodate were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents used were of analytical grade.

Materials. Artichoke was bought fresh in the town market (Madrid, Spain). The edible portion (heads) was separated by cutting leaves and outer bracts and was freeze-dried immediately. It was then ground (0.5 mm mesh) and stored at −18 °C until analysis.

Animals and Diets. Male Wistar rats (n = 20; 170−180 g), 8 weeks of age, were from the feeding animal center of the Institute of Nutrition and Bromatology, CSIC (Madrid, Spain). The use of animals was conducted in compliance with the European guidelines for the care and use of laboratory animals. The rats were housed individually in wire cages in a room maintained at 23 ± 2 °C, with a controlled 12-h light−dark cycle. All rats had free access to food and tap water. Body weight and food intakes were recorded at the end of the experiment. After 3 weeks rats were sacrificed by decapitation. Rats were randomly assigned to two groups. One group was fed a diet containing artichoke (∼138 g/kg of diet) and the other group a basal diet of AIN-93M Purified Rodent Diet (Dyets, Inc.). This diet is specially formulated to provide enough energy and nutrients (*16*). Both control and test diet contained 80 g/kg indigestible fraction (*17*), which comprises not only dietary fiber but also other compounds of proven resistance to the action of digestive enzymes such as a fraction of resistant starch, protein, certain polyphenols and other associated compounds. The compositions of the two diets are shown in **Table 1**. Both diets were balanced in energy using cornstarch. The approximate composition of edible artichoke is indicated in **Table 2**.

Blood Sampling and Preparation. Blood samples were collected in EDTA-coated tubes and centrifuged at 1500g for 10 min at 4 °C. The plasma and erythrocytes were stored separately at −80 °C until analysis. Erythrocytes were resuspended in 1 volume of distillate water for lysis. The clear hemoglobin (Hb) layer was used for the analyses of Hb oxidation and antioxidant enzymes.

Table 2. Approximate Composition of Artichoke (Grams per 100 g of Edible Portion, Dry Matter)[a]

protein	18.91 ± 0.5
lipids	1.69 ± 0.11
soluble carbohydrates	12.41 ± 1.12
ash	9.31 ± 0.59
indigestible fraction	
soluble	13.47 ± 1.73
insoluble	44.42 ± 1.91
extractable polyphenols	5.04 ± 0.16

[a] Values are means ± standard deviation of three replicate determinations.

Indigestible Fraction (IF). The method described by Saura-Calixto et al. (*17*) was followed to determine the IF content in globe artichoke and experimental diets. Enzymatic treatment with pepsin and α-amylase was carried out in order to eliminate digestible components of the sample. Samples were centrifuged and supernatants removed. Residues were washed twice with distilled water, and the residues were dried overnight at 105 °C and quantified gravimetrically as the insoluble IF. Supernatants were transferred into dialysis tubes (12000−14000 MWCO; Dialysis Tubing Visking, Medicell International Ltd., London, U.K.) and dialyzed against water for 48 h at 25 °C (water flow = 7 L/h). Dialysis retentates were then hydrolyzed with 1 mol/L sulfuric acid at 100 °C for 90 min, and the soluble IF was measured with dinitrosalicylic acid (*18*).

Extraction of Polyphenols. One gram of ground, freeze-dried artichoke sample was placed in a test tube; 40 mL of methanol/water (50:50) and sufficient HCl to obtain a final pH 2.0 were added, and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500g for 10 min, and the supernatant was recovered. Forty milliliters of acetone/water (70:30) was added to the residue, and shaking and centrifugation were repeated. Both extracts were mixed. Extracts were produced in triplicate and used to measure the total polyphenol (TP) content and the antioxidant capacity. TP of the extracts was determined according to the Folin−Ciocalteu procedure (*19*), using gallic acid as standard and expressing the results as gallic acid equivalents (GAE).

Measurement of Antioxidant Activity. *In Vitro Determinations. (a) DPPH$^{\bullet}$ Free Radical Scavenging Assay.* The antioxidant activity of aqueous−methanol−acetone artichoke extract was measured in terms of radical scavenging ability, according to the DPPH$^{\bullet}$ method (*20*). The parameter EC_{50}, which reflects the depletion of DPPH$^{\bullet}$ free radical to 50%, was expressed in terms of Trolox equivalents per gram of artichoke in the reaction medium (*21*). The time taken to reach the steady state at EC_{50} (T_{EC50}) was calculated. The antiradical efficiency (AE), a parameter that combined both factors ($1/EC_{50} \times T_{EC50}$), defined by Sánchez-Moreno et al. (*20*), was also calculated to recognize both effects of the aforementioned parameters.

(b) FRAP Assay. The antioxidant capacity of aqueous−methanol−acetone artichoke extract is estimated according to the procedure described by Benzie et al. (*22*), with some modifications introduced in our laboratory (*23*). Briefly, 900 μL of FRAP reagent, freshly prepared and warmed at 37 °C, was mixed with 90 μL of distilled water and 30 μL of either test sample, standard, or appropriate reagent blank. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$, plus 25 mL of 0.3 mol/L acetate buffer, pH 3.6.

Readings at the absorption maximum (595 nm) were taken every 15 s using a BD spectrophotometer. Temperature was maintained at 37 °C. The readings at 4 and 30 min were selected for calculation of FRAP values. Methanolic solutions of known Trolox concentrations were used for calibration.

(c) Copper-Induced Oxidation of Human LDL Assay. The antioxidant capacity of the aqueous−methanol−acetone artichoke extract in terms of protection of LDL against in vitro copper-induced oxidation was measured. The blood was collected from a patient with homozygous familial hypercholesterolemia from Ramón y Cajal Hospital, Madrid, Spain. LDL was isolated following the procedure of Tani (*24*) and the

Table 3. Total Phenolic Content (TP) Expressed as Gallic Acid Equivalents (GAE), Free Radical DPPH• Scavenging Activity, Ferric-Reducing Antioxidant Power (FRAP), and Protection against Oxidation in Cu-Induced LDL System Expressed as Trolox Equivalents of Artichoke Aqueous Methanol Extracts[a]

TP (mg of GAE/g of dm)	EC_{50} (μmol of TE/g of dm)	DPPH•		FRAP (μmol of TE/g of dm)		Cu-induced LDL oxidation
		T_{EC50} (min)	AE (×10^4)	4 min	30 min	CLT_{50} (μmol of TE/g of dm)
50.04 ± 0.9	128 ± 3	27.67 ± 3.1	2.89 ± 0.1	235 ± 15	343 ± 25	1150 ± 68

[a] Each value is the mean ± standard deviation of three replicate experiments. For calculation see Materials and Methods.

experimental procedure performed as described previously (*25*). Maximal time (T_{max}), which is the time in minutes needed to reach the maximum amount of dienes, was also calculated. Finally, the parameter CLT_{50}, introduced by Sánchez-Moreno et al. (*25*), was also calculated to measure the concentration of antioxidant that increases the lag time to 50% greater than that of the control, eliminating the LDL status influence.

Ex Vivo Determinations in Rats. (a) FRAP Assay. The antioxidant capacity of plasma was estimated according to the procedure described above. This assay was performed in 10 animals from each group.

We calculated the theoretical contribution of the plasma uric acid (UA) levels in the different rats by using the dose−response curves of UA concentration (range = 40−1000 μmol/L) versus FRAP value. These calculated contributions were subtracted from the corresponding experimental total FRAP value, resulting in the $FRAP_{-AU}$ 4 min and $FRAP_{-AU}$ 30 min values.

(b) ABTS Assay. The antioxidant capacity was estimated following the procedure described by Re et al. (*26*). ABTS radical cation ($ABTS^{•+}$) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12−16 h before use. The $ABTS^{•+}$ solution (2 days stable) was diluted with PBS to an absorbance of 0.70 ± 0.02 at 730 nm. After the addition of 10 μL of sample or Trolox standard to 4 mL of diluted $ABTS^{•+}$ solution, absorbance readings were taken every 20 s using a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). The reaction was monitored during 6 min. The percentage inhibition of absorbance versus time was plotted, and the area below the curve (0−6 min) was calculated. Ethanolic solutions of known Trolox were used for calibration. This assay was performed in 10 animals from each group.

(c) Determination of 2-Aminoadipic Semialdehyde (2-AAS). This procedure was performed as previously described by Daneshvar et al. (*27*). Briefly, a 0.1 mL plasma sample was mixed with 0.1 mL of 0.25 mol/L (*N*-morpholino)ethanesulfonic acid (MES) buffer, pH 6.0. A solution of 0.25 mol/L MES, containing 1% SDS (w/v), was added (0.5 mL), followed by 50 μL of 0.25 mol/L fluoresceinamine in 0.52 mol/L NaOH and 50 μL of 0.25 mol/L $NaCNBH_3$ in 0.25 mol/L MES, pH 6.0, and the mixture was incubated for 1 h at 37 °C and added to 2 mL of deionized water. The same procedure was followed in the case of erythrocytes lysates, without the initial addition of 0.25 mol/L MES. The mixture was applied to a Sephadex G-25 gel filtration column, and the column was eluted with water. The protein fraction (3.5 mL) was collected and precipitated by the addition of 1 mL of 70% trichloroacetic acid. The precipitate was hydrolyzed in 1 mL of 6 mol/L HCl at 110 °C for 24 h. The hydrolysate was filtered through cellulose triacetate filters (Vectaspin micro, Whatman, Maidstone, U.K.) and 50 μL injected into an HPLC with an ODS column (2.1 × 200 mm, 5 μm, Hewlett-Packard) eluted by a gradient of acetonitrile in 0.5 mol/L ammonium acetate, pH 6.5. The acetonitrile gradient program was 0% (3 min hold), increased to 15% (at 8 min), to 35% (12 min), and to 100% (17 min). The eluate was monitored at 454 nm, the absorption maximum of decarboxylated fluoresceinamine, and at 275 nm for determination of free L-tyrosine, a marker for the quantity of hydrolyzed protein. The results were expressed as picomoles of 2-AAS per milligram of protein. These assays were performed in five animals from each group.

(d) Antioxidant Enzymes. Automated assays were performed on a Cobas Mira analyzer (Roche, Basel, Switzerland) to determine the activities of the antioxidant enzymes superoxide dismutase (EC 1.1.5.1; SOD), glutathione peroxidase (EC 1.11.1.9; Gpx), glutathione reductase (EC 1.6.4.1; GR), and catalase (EC 1.11.1.6; CAT) in erythrocyte lysates. The activity of the enzymes was related to the amount of Hb in the blood samples. SOD (Randox catalog no. SD 125), Gpx (Randox catalog no. RS 505), and Hb (Randox catalog no. HG 980) were determined using commercially available kits. GR activity and CAT activity were determined according to the methods described previously (*28*). These assays were performed in five animals from each group.

Statistical Analysis. Results are expressed as mean values ± standard deviation (SD). Comparison of means of three measurements, using a significance level of $P < 0.05$, was performed by one-way analysis of variance (ANOVA). Statgraphic Computer System, version 5.1, was used.

RESULTS AND DISCUSSION

In Vitro Antioxidant Activities. The generation of radical oxidative species involves radical processes generated either by inorganic factors or by different biological redox systems (*29*). The partition of antioxidant compounds determines their effective antioxidant activity in either aqueous or lipid systems (*30*). Therefore, to evaluate the antioxidant activity of the artichoke extracts, two aqueous-based models, which measure, respectively, the radical scavenging activity and the total reduction power, were chosen. In addition, to evaluate the antioxidant activity in a lipophilic medium, copper-induced oxidation of lipoprotein model was selected to measure the prevention of lipid peroxidation or metal-catalyzed radical reactions.

The parameter EC_{50}, which reflects the depletion of DPPH• free radical to 50% from aqueous−organic artichoke extract, the time taken to reach the steady state at EC_{50} (T_{EC50}), the parameter antiradical efficiency (AE), and the total phenolic content estimated by Folin−Ciocalteu, are shown in **Table 3**. The phenolic values estimated for the edible portion of artichoke give a range of 31−58 mg/g GAE dry matter (dm) (*31*), in which our estimated value (50.04) is included. Chlorogenic acid (3-caffeoylquinic acid), cynarin (1,5-dicaffeoylquinic acid), luteolin, and luteolin glycosides are considered to be essential artichoke compounds (*6*, *11*, *32*).

The antioxidant potential of the artichoke aqueous−methanol extracts was estimated from their ability to reduce the TPTZ−Fe(III) complex to TPTZ−Fe(II) complex (**Table 3**).

Artichoke samples were tested in a system containing human LDL, which was oxidized in vitro by copper. It is clear from these experiments that the artichoke aqueous−methanol extract prolongs the lag phase in a concentration-dependent manner ($r = 0.9680$, $P < 0.05$). Also, the maximal time (T_{max}) of this oxidation reaction was dependent on the concentration of added antioxidant extract ($r = 0.9643$, $P < 0.05$). The lag phase of the LDL oxidation reaction is dependent on the physiological natural LDL content of the liposoluble antioxidative compounds, such as α-tocopherol or carotenoids (*33*). The antioxidant effect of artichoke extracts was studied in a system containing 50 μg of protein/mL of human LDL, which was oxidized in vitro by 5 μmol/L $CuCl_2$. The CLT_{50} value is shown in **Table 3**.

Table 4. Effect of 3 Week Artichoke Intake in Rat on Plasma Biomarkers for Oxidative Defense (Median Values and 99% Confidence Interval in 10 Rats from Each Group)[a]

	control group		exptl group		difference	
	mean	range	mean	range	mean	range
FRAP 4 min (μmol of TE/L)	178	94–254	147	101–216	31.0 s	7.3, 38
FRAP 30 min (μmol of TE/L)	315	200–464	321	214–401	5.40 ns	13, 63
$FRAP_{-AU}$ 4 min (μmol of TE/L)	58	0.1–112	102	5.0–163	44.0 s	50, 51
$FRAP_{-AU}$ 30 min (μmol of TE/L)	254	136–406	280	152–406	26.0 ns	16, 0.0
ABTS (μmol of TE/L)	5317	4489–5903	5036	4284–5671	281 ns	205, 232
UA (μmol/L)	175	130–226	78.5	41.6–136	–97.0 s	–89.3, –89.2

[a] s = significant; ns = not significant.

Our group has found edible artichoke to be one of the best vegetable antioxidant sources in the Spanish diet (*34*). With regard to artichoke in vitro antioxidant studies, Wang et al. (*35*) report a wide range of relative activities toward DPPH• (8.3−49.7%), which are correlated with their polyphenol contents (1.7−9.86% dm) of the different artichoke samples used (r = 0.96). Other authors (*36*) showed a relatively high free radical scavenging activity (versus both DPPH• and $ABTS^{•+}$ radicals) as well as capacity to inhibit lipid peroxidation (ferric thiocyanate method) in artichoke byproducts.

One gram (dm) had a DPPH• activity (*22*) and a FRAP value (*24*) equivalent to those of 29.2 and 62.6 mg of vitamin C and to those of 77.9 and 159 mg of vitamin E, respectively. Thus, edible artichoke could contribute to the recommended dietary intake of these vitamins.

It must be borne in mind that in vitro activities can be considered only potentially relevant in biological systems and that in vivo activities depend also on bioavailability and biotransformation. It is known that chlorogenic and caffeic acids, which are contained in artichoke, are partially bioavailable (*37*) and, consequently, their in vivo activities could be significant. The protective effects of artichoke require further examination. Especially, the isolation, identification, and quantification of certain flavonoids and hydroxycinnamic acids from artichoke should be emphasized. Moreover, the metabolic fate of these compounds is important for the evaluation of their efficiency in the human body.

Antioxidant Status Evaluation. The antioxidant system in plasma and erythrocytes is constituted by enzymatic and nonenzymatic antioxidants, and it is difficult to evaluate each nonenzymatic antioxidant separately. Moreover, an isolated measure of one type of antioxidant does not take into account the potential synergic effects among antioxidants. Thus, the measure of a unique antioxidant could be less representative than the global antioxidant measure. As a consequence, we applied different methodologies to evaluate the two biological systemic compartments: plasma and erythrocytes.

A significant difference in the daily food intake and body weight gain between the experimental (artichoke) and control groups was found, these parameters being higher in the case of the animals not fed with artichoke. However, the food efficiency (FE), a parameter that related both parameters (FE = body weight gain/food intake), did not show any significant difference between the tested groups. Thus, it seems that the former parameters do not affect the data interpretation of the antioxidative status of the animals.

Apparently, the intake of artichoke did not lead to an increase in the value of the ferric reduction capacity in plasma, neither in the measurement at 4 min nor at 30 min (**Table 4**). Also, ABTS radical scavenging activity was not modified in the control group with respect to the artichoke-fed group (**Table 4**).

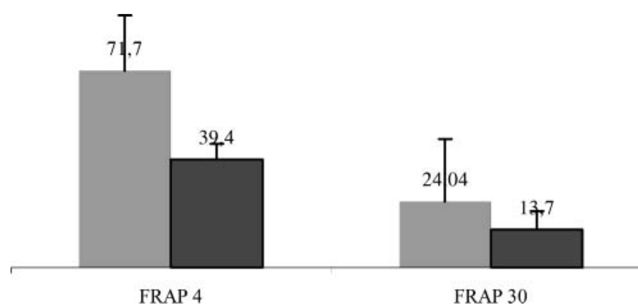

Figure 1. Plasma uric acid contribution (percent) to FRAP 4 min and FRAP 30 min values: (lightly shaded bars) control; (heavily shaded bars) artichoke.

However, uric acid (UA), which is the major contributor to the total FRAP value in human blood (*22*), was depleted in the artichoke-fed group versus the control group (55% reduction on average) (**Table 4**). The $FRAP_{-AU}$ 4 min and $FRAP_{-AU}$ 30 min values are given in **Table 4**. There were significant differences between the artichoke-fed and control rats in $FRAP_{-AU}$ 4 min, but not in $FRAP_{-AU}$ 30 min. In the case of UA, the relationship of FRAP 4 min/FRAP 30 min is absolutely lineal (slope = 1.0197, $P < 0.000$). This means that UA reacts completely with the ferric system immediately after the reaction is started, and as a consequence UA at 4 min has the same FRAP value as at 30 min. Thus, UA reacts immediately in the FRAP assay, whereas other antioxidants react more slowly. This reveals that whereas artichoke feeding leads to a decrease in plasma UA, this is compensated by an increase in other slow-reacting antioxidants leading to a steady state in total FRAP and in $FRAP_{-AU}$ 30 min (**Figure 1**).

It has been suggested that a low human intake of fruits and vegetables rich in polyphenols and carotenoids could exert an effect in the erythrocyte activity of antioxidant enzymes (*38*). The results of the measures on the antioxidant enzymes in both animal groups tested are shown in **Table 5**. A significant difference between the artichoke and control groups was found for the Gpx activity only. Thus, the intake of artichoke during 3 weeks modulated the AS of erythrocytes. The protective effect of artichoke could be responsible for a decrease in the Gpx degradation, leading to a relative increase in the activity of the enzyme in the artichoke group. An alternative explanation could be that artichoke induces the synthesis of this enzyme in the red bone marrow, leading to increased levels in erythrocytes (*39*). A similar effect on erythrocyte Gpx activity has been observed in humans after the intake of a diet supplemented with apple and black currant juices (*40*) or with grape skin extract (*41*). Bobek et al. (*42*) performed a hypercholesterolemic rat study in which animals were fed with a diet supplemented with tomato, apple, or grape pomaces. In all of the supplemented diets decreases in SOD, CAT, and Gpx enzymes (range = 36−56%) were observed. In our study with normocholesterolemic

Table 5. Effect of 3 Week Artichoke Intake in Male Rat on Blood Biomarkers for Oxidative Defense (Median Values and 99% Confidence Interval in Five Rats from Each Group)[a]

	control group		exptl group		difference	
	mean	range	mean	range	mean	range
plasma AAS (pmol/protein)	165	155–175	148	136–160	−17 s	−19, −15
RBC AAS (pmol/mg of Hb)	64	63–65	52	47–59	−12 s	−16, −6
RBC SOD (units/g of Hb)	19259	7367–35059	11469	15481–29701	−7790 ns	+8114, −5358
RBC Gpx (units/g of Hb)	7586	5486–10232	16836	12371–20373	9250 s	6.885, 10141
RBC GR (units/g of Hb)	32	24–37	22	16–26	−10 ns	−7.6, −11
RBC CAT (units/g of Hb)	129	107–160	93	54–124	−36 ns	−52, −35

[a] s = significant; ns = not significant.

rats, the intake of artichoke modulated the activity of Gpx. A relationship between cholesterol levels and antioxidant enzyme activities has not yet been investigated, and more research has to be done to explain this apparent discrepancy. Maybe the Hb levels are increased in the mentioned study, leading to an artifact result.

Protein oxidation seems to play a role in the development of certain degenerative diseases such as atherosclerosis, diabetes, cataracts, and neurologic diseases (*43*−*47*). Recently, specific products of protein oxidation have been assessed in animal studies. The biomarker of protein oxidation, 2-AAS, increased in rats dosed with the pro-oxidants, acrolein, or *tert*-butylhydroperoxide (*27*). Other specific oxidation products have been increased in rats in which stress was induced by exercise (*48*), hypoxia (*49*), or chemical agents (*50*). In all of these works the use of a unique specific biomarker of protein is suggested as an efficient measure of the whole protein oxidation. In the present study 2-AAS significantly decreased in plasma and in erythrocytes in the artichoke group compared to the control group (**Table 5**). This suggests that artichoke confers protection against protein oxidation in the blood. It is possible that erythrocytes are generators of reactive oxygen species (ROS) due to the presence of iron and that ROS leak to plasma. It could be hypothesized that artichoke protection in the erythrocyte compartment may contribute to the protection effect in plasma, decreasing the effect of ROS in the plasma compartment. Bioactive compounds in artichoke, such as flavones or hydroxycinnamic acids, might alternatively protect the protein from oxidation both in plasma and in erythrocytes. Previous studies in rodent models suggest a protection by the flavonoids quercetin or epicatechin in plasma (*51*, *52*).

In conclusion, the in vitro protective activity of artichoke was confirmed in a rat model. AS in plasma compartment determined by changes in $FRAP_{-AU}$ and 2-AAS seems to be influenced by AS in erythrocyte, possibly due in part to an increased activity of Gpx.

ABBREVIATIONS USED

ABTS, 2,2′-azinobis(3-ethylbenzothiazolin-6-sulfonate); ANOVA, one-way analysis of variance; 2-AAS, 2-aminoadipicsemialdehyde; AE, antiradical efficiency; AS, antioxidant status; CAT, catalase; dm, dry matter; $DPPH^•$, 2,2-diphenyl-1-picrylhydrazyl; Hb, hemoglobin; T_{max}, maximal time; CLT_{50}, concentration of antioxidant that increases the lag time to 50% greater than that of the control; EC_{50}, amount of sample necessary to decrease by 50% the initial $DPPH^•$ concentration; EDTA, ethylenediaminetetraacetic acid; FRAP, ferric-reducing antioxidant power; Gpx, glutathione peroxidase; GR, glutathione reductase; IF, indigestible fraction; LDL, low-density lipoprotein; RBC, red blood cells; ROS, reactive oxygen species; SOD, superoxide dismutase; T_{EC50}, time taken to reach the steady state at EC_{50}; TP, total polyphenols; TPTZ, 2,4,6-tri(2-pyridyl)-*s*-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UA, uric acid.

LITERATURE CITED

(1) Halliwell, B. Antioxidants and human disease: A general introduction. *Nutr. Rev.* **1997**, *55*, S44−S52.

(2) Temple, N. J. Antioxidants and disease: More questions than answers. *Nutr. Res.* **2000**, *2*, 449−459.

(3) Alcachofa. In *Anuario de Estadística Agraria*; Ministerio de Agricultura y Pesca: Madrid, Spain, 1997; p 244.

(4) Adzet, T.; Camarasa, J.; Laguna, C. J. Hepatoprotective activity of polyphenolic compounds from *Cynara scolymus* against CCl_4 toxicity in isolated rate hepatocytes. *J. Nat. Prod.* **1987**, *50*, 612−617.

(5) Gebhardt, R. Antioxidative and protective properties of extracts from leaves of the artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* **1997**, *144*, 79−286.

(6) Pinelli, P.; Romani, A.; Galardi, C.; Mulinaci, N.; Cimato, A.; Coinu, R.; Franconi, F. Characterization of artichoke extracts and evaluation of their antioxidant activity. In *Polyphenols Communications*; El Hadrami, I., Ed.; Abstracts of the XXI International Conference on Polyphenols El Watania: Marrakech, Morocco, Sept 9−12, 2002; pp 577−578.

(7) Williamson, G. Protective effects of fruits and vegetables in the diet. *Nutr. Food Sci.* **1996**, *1*, 6−10.

(8) Frankel, E. N.; Meyer, A. S. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925−1941.

(9) Moure, A.; Cruz, J. M.; Franco, D.; Domínguez, J. M.; Sineiro, J.; Domínguez, H.; Nuñez, M. J.; Parajó, J. C. Natural antioxidants from residual sources. *Food Chem.* **2001**, *72*, 145−171.

(10) Schwarz, K.; Bertelsen, G.; Nissen, L. R.; Gardner, P. T.; Heinonen, M. I.; Hopia, A.; Huynh-Ba, T.; Lambelet, P.; McPhail, D.; Skisbsted, L. H.; Tijburg, L. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal compounds. *Eur. Food Res. Technol.* **2001**, *212*, 319−328.

(11) Brown, J. E.; Rice-Evans, C. A. Luteolin-rich artichoke extract protects low-density lipoprotein from oxidation *in vitro*. *Free Radical Res.* **1998**, *29*, 247−255.

(12) Prior, R. L.; Cao, G. *In vivo* antioxidant capacity: Comparison of different analytical methods. *Free Radical Biol. Med.* **1999**, *27*, 1173−1181.

(13) Halliwell, B. Establishing the significance and optimal intake of dietary antioxidants: The biomarker concept. *Nutr. Rev.* **1999**, *57*, 104−113.

(14) Fahey, J.; Beverly, M. S.; Clevidence, A.; Russell, R. M. Methods for assessing the biological effects of specific plant components. *Nutr. Rev.* **1999**, *57*, S43−S40.

(15) Zwart, L. L.; Meerman, J. H. N.; Commandeur, J. N. M.; Vermeulen, N. P. E. Biomarkers of free radical damage applications in experimental animals in humans. *Free Radical Biol. Med.* **1999**, *26*, 202−226.

(16) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.

(17) Saura-Calixto, F.; García-Alonso, A.; Goñi, I.; Bravo, L. In vitro determination of the indigestible fraction in foods: An alternative to dietary fiber analysis. *J. Agric. Food Chem.* **2000**, *48*, 3342−3347.

(18) Englyst, H. N.; Cummings, J. H. Improved method for the measurement of dietary fibre as non-starch polysaccharide in plant foods. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 808−814.

(19) Montreau, F. R. Sur le dosage des composés phénoliques totaux dans les vins par la methode Folin−Ciocalteu. *Connaiss. Vigne Vin* **1972**, *24*, 397−404.

(20) Sánchez-Moreno, C.; Larrauri, J. A.; Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* **1998**, *76*, 270−276.

(21) Jiménez-Escrig, A.; Jiménez-Jiménez, I.; Pulido, R.; Saura-Calixto, F. Antioxidant activity of fresh and processed edible seaweeds. *J. Sci. Food Agric.* **2001**, *81*, 500−504.

(22) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(23) Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **2000**, *47*, 3396−3402.

(24) Tani, N. Development of selective low-density lipoprotein (LDL) apheresis system: immobilized polyanion as LDL-specific adsorption for LDL apheresis system. *Artif. Organs* **1995**, *20*, 922−929.

(25) Sánchez-Moreno, C.; Jiménez-Escrig, A.; Saura-Calixto, F. Study of low-density lipoprotein oxidizability indexes to measure the antioxidant activity of dietary polyphenols. *Nutr. Res.* **2000**, *20*, 941−954.

(26) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231−1237.

(27) Daneshvar, B.; Frandsen, H.; Autrup, H.; Dragsted, O. γ-glutamyl semialdehyde and 2-aminoadipic semialdehyde: biomarkers of oxidative damage to proteins. *Biomarkers* **1997**, *2*, 117−123.

(28) Wheeler, C. R.; Salzman, J. A.; Elsayed, N. M.; Omayer, S. T.; Korte, D. W. Automated assay for superoxide dismutase, catalase glutathione peroxidase, and glutathione reductase. *Anal. Biochem.* **1990**, *184*, 193−199.

(29) Jacob, R. A. The integrated antioxidant system. *Nutr. Res.* **1995**, *15*, 755−766.

(30) Krinsky, N. I. Mechanisms of action of biological antioxidants. *Proc. Soc. Exp. Biol. Med.* **1992**, *200*, 248−254.

(31) Lattanzio, V.; Sumere van, C. F. Changes in phenolic compounds during the development and cold storage of artichoke (*Cynara scolymus* L.) heads. *Food Chem.* **1987**, *24*, 37−50.

(32) Lattanzio, V.; Cardinali, A.; Venere di, D.; Linslata, V.; Palmieri, S. Browning phenomena in stored artichoke (*Cynara scolymus* L.) heads: enzymic or chemical reactions? *Food Chem.* **1994**, *50,* 1−7.

(33) Esterbauer, H.; Striegel, G.; Puhl, H.; Rotheneder, M. Continuous monitoring of *in vitro* oxidation of human low-density lipoprotein. *Free Radical Res. Commun.* **1989**, *6,* 67−65.

(34) Jiménez-Escrig, A. Evaluación de la capacidad antioxidante de alimentos vegetales. Ph.D. Thesis, Universidad Complutense de Madrid, Madrid, Spain, 2001.

(35) Wang, M.; Simon, J. E.; Aviles, I. F.; He, K.; Zheng, Q.-Y.; Tadmor, Y. Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *J. Agric. Food Chem.* **2003**, *51*, 601−608.

(36) Llorach, R.; Espín, J. C.; Tomás-Barberán, F. A.; Ferreres, F. Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *J. Agric. Food Chem.* **2002**, *50*, 3458−3464.

(37) Olthof, M. R.; Hollman, P. C. H.; Katan, M. B. Cholorgenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **2001**, *131*, 66−71.

(38) Nielsen, S. E.; Young, J. F.; Daneshvar, B.; Lauridsen, S. T.; Knuthsen, P.; Sandstrom, B.; Dragsted, L. O. Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. *Br. J. Clin. Nutr.* **1999**, *81*, 447−455.

(39) Castenmiller, J. J. M.; Lauridsen, S. T.; Dragsted, L. O.; Hof van het, K. H.; Linssen, J. P. H.; West, C. E. β-carotene does not change markers of enzymatic and nonenzymatic antioxidant activity in human blood. *J. Nutr.* **1999**, *129*, 2162−2169.

(40) Young, J. F.; Nielsen, S. E.; Haraldsdöttir, J.; Daneshavar, B.; Lauridsen, S. T.; Knuthsen, P.; Crozier, A.; Sandström, B.; Dragsted, L. O. Effect of juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *Am. J. Clin. Nutr.* **1999**, *69*, 87−84.

(41) Young, J. F.; Dragsted, L. O.; Daneshavar, B.; Lauridsen, S. T.; Hansen, M.; Sandström, B. The effect of grape-skin extract on oxidative status. *Brit. J. Nutr.* **2000**, *84*, 505−513.

(42) Bobek, P.; Ozdin, L.; Hromadova, M. The effect of dried tomato, grape and apple pomace on the cholesterol metabolism and antioxidative enzymatic system in rats with hypercholesterolemia. *Nahrung* **1998**, *42*, 317−320.

(43) Dean, R. T.; Shanlin, F. U.; Stocker, R.; Davies, M. J. Biochemistry and pathology of radical-mediated protein oxidation. *Biochem. J.* **1997**, 324, 1−18.

(44) Grune, T.; Reinheckel, T.; Davies, K. J. A. Degradation of oxidized proteins in mammalian cells. *FASEB J.* **1997**, *11*, 526−534.

(45) Onorato, J. M.; Thorpe, S. R.; Baynes, J. W. Immunohistochemical and ELISA assays for biomarkers of oxidative stress in aging and disease. *Ann. N. Y. Acad. Sci.* **1998**, *854*, 277−290.

(46) Gracy, R. W.; Talent, J. M.; Kong, Y.; Conrad, C. C. Reactive oxygen species: the unavoidable environmental insult? *Mutat. Res* **1999**, *428*, 17−22.

(47) Suzuki, D.; Myata, T. Carbonyl stress in the pathogenesis of diabetic nephhropathy. *Intern. Med.* **1999**, *38*, 309−314.

(48) Liu, J.; Teo, H. C.; Overvik-Douki, E.; Hagen, T.; Doniger, S. J.; Chu, D. W.; Brooks, G. A.; Ames, B. N. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J. Appl. Physiol.* **2000**, *89*, 21−28.

(49) Awasthi, S.; Gyurasics, A.; Knight, S. A.; Welty, S. E.; Smith, C. Y. Protein oxidation biomarkers in hyperoxic lung injury in rats, effects of U-74389. *Toxicol. Lett.* **1998**, *95*, 47−61.

(50) Lee, S. K.; Lee, B. M. Oxidation of erythrocyte protein and lipid, and haemolysis in rabbit red blood cells treated with benzo[*a*]-pyrene or adriamycin. *J. Toxicol. Environ. Health* **1997**, *51*, 557−569.

(51) Morand, C.; Crespy, V.; Manach, C.; Besson, C.; Demigne, C.; Remesy, C. Plasma metabolites of quercetin and their antioxidant properties. *Am. J. Physiol.* **1998**, *275,* R212−R219.

(52) Terao, J. Dietary flavonoids as antioxidants in vivo: conjugated metabolites of epichatechin and quercetin participate in antioxidative defense in blood plasma. *J. Med. Invest.* **1999**, *46*, 159−168.

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