JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Wild Artichoke Prevents the Age-Associated Loss of Vasomotor Function

GIUSEPPE ROSSONI, SIMONA GRANDE, CLAUDIO GALLI, AND FRANCESCO VISIOLI*

Department of Pharmacological Sciences, University of Milan, Milan 20133, Italy

Endothelial dysfunction, which is more often observed in conduit arteries such as the aorta, carotid, femoral, and brachial arteries, is largely due to alterations in cellular signal transduction initiated by an escalating cycle of damage triggered by oxidative stress. This phenomenon is exacerbated in the elderly, where a progressive loss of vascular endothelial function and concurrent loss of vasomotor control is frequent. In a previous study, we demonstrated that the wild artichoke (*Cynara cardunculus*) is able to increase the production of the vasorelaxant factor nitric oxide by cultured aortic endothelial cells. We now extended that study to verify (1) the vasorelaxant potential of *C. cardunculus* on isolated rat aortic rings and (2) whether the vasomodulating properties of *C. cardunculus* are maintained *in vivo*, after administration to aged rats. The results demonstrate that the wild artichoke and its main components, namely, luteolin and apigenin, improve aortic relaxation when added to the incubation bath. Moreover, the feeding of wild artichoke [10 mg (kg of polyphenols)$^{-1}$ day^{-1}] to aged rats significantly restores proper vasomotion, to a degree similar to that observed in young animals. This study provides further justification to the advice to consume wild greens as part of a balanced diet and suggests that close attention should be paid to the diet of the elderly, because it can effectively modulate important parameters of cardiovascular risk.

KEYWORDS: Mediterranean diet; *Cynara cardunculus*; vasorelaxation; antioxidants; aging

INTRODUCTION

Endothelial dysfunction is more often observed in conduit arteries (such as the aorta, carotid, femoral, and brachial arteries) and is largely due to alterations in cellular signal transduction initiated by an escalating cycle of damage triggered by oxidative stress (*1*, *2*). The latter reduces production/availability of the vasorelaxant factor nitric oxide (NO) (*1*, *3*). This phenomenon is exacerbated in the elderly, where a progressive loss of vascular endothelial function and resultant loss of vasomotor control is frequent (*4*).

The incidence of coronary heart disease (CHD) is low in the Mediterranean area, in part because of the high intake of plant foods, either in the form of complex carbohydrates or as fresh vegetables, spices, legumes, and fruits; consumption of olive oil as the principal source of fat; concomitant low intake of saturated fat; and moderate consumption of wine with meals (*5*). Plants' products of secondary metabolism, in particular flavonoids and polyphenols, are endowed with "pharmacological" activities that might favorably affect human health and lower the risk for CHD (*6*). This holds particularly true in areas where most of the intake of polyphenols and flavonoids derives from local wild plants (*7*, *8*).

In a previous study, we demonstrated that extracts of wild artichoke (*Cynara cardunculus*) and wild thyme (*Thymus pulegioides*) are able to increase NO production by cultured aortic endothelial cells (*9*). We now extended that study to verify (1) the vasorelaxant potential of *C. cardunculus* on isolated rat aortic rings and (2) whether the vasomodulating properties of *C. cardunculus* are maintained *in vivo*, after administration to aged rats, because the most powerful independent predictor for incident cardiovascular disease is, indeed, advancing age (*10*). Moreover, we investigated the effects of *C. cardunculus* administration on surrogate markers of CHD such as blood thrombogenicity and *in vivo* oxidative stress.

MATERIALS AND METHODS

All procedures involving animals and their care were conducted at the Department of Pharmacological Science, University of Milan, in conformity with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH publication number 85-23, revised 1996).

Preparation of a *C. cardunculus* Extract. The dried leaves of wild artichoke (*C. cardunculus*), collected in Castelmezzano (Italy) (*8*, *11*), were used for the preparation of a water-soluble extract. A stock solution was prepared by dispersing 10 g in 1 L of distilled water at pH 8.5. The mixture was gently stirred at 100 °C for 15 min; the extract was filtered through filter paper; and the pH was adjusted to 6.0−6.5. Finally, the volume was reduced under nitrogen, and the aqueous extract was stored at −20 °C in the dark.

Determination of Polyphenolic Content. The total polyphenolic content of the *C. cardunculus* extract was determined colorimetrically

* To whom correspondence should be addressed: Department of Pharmacological Sciences, Via Balzaretti 9, University of Milan, Milan 20133, Italy. Telephone: +39-0250318280. Fax: +39-02700426106. E-mail: francesco.visioli@unimi.it.

10.1021/jf052499s CCC: $30.25
Published on Web 12/06/2005

by the Folin−Ciocalteau method, using gallic acid as the reference compound (*12*).

HPLC Analysis of *C. cardunculus*. Analytical separations were carried out on a 250 × 4.6 mm stainless-steel C_{18} column (Waters, Wexford, Ireland) coupled with a Jasco 875 UV detector (*9*). Identification of individual phenolics (namely, luteolin and apigenin) was performed by comparison with authentic standards.

***In Vitro* Vasomodulating Effect of *C. cardunculus*.** Aortic rings were obtained from male Sprague−Dawley rats (200−250 g; Charles River Laboratories, Calco, Italy). Briefly, after the removal of the superficial connective tissue, the aorta was cut into ring segments, 3−4 mm in width, which were then mounted in standard 5-mL organ baths filled with Krebs Henseleit solution, whose composition was (in millimolar) NaCl, 118.0; KCl, 2.8; KH_2PO_4, 1.2; $MgSO_4$, 1.2; (+)-glucose, 5.0; $NaHCO_3$, 25.0; and $CaCl_2$, 2.5, containing ethylenediaminetetraacetic acid (0.026 mM) and ascorbic acid (0.1 mM) to prevent oxidation of flavonoids. The bath medium (pH 7.4) was maintained at 37 °C and was continuously aerated with 95% O_2 and 5% CO_2. When required, the endothelium was mechanically removed in some rings by gently rubbing the lumen with the tips of fine forceps. Tissues were connected via silk sutures to force-displacement transducers (model 7004; U. Basile, Comerio, Varese, Italy), and changes in isometric force were displayed on a Gemini chart recorder (model 7070; U. Basile). All rings were gradually stretched to a basal resting tension of 1.2−1.5 g, which was maintained throughout the experiment. The preparations were allowed to equilibrate for 90 min.

To evaluate maximal contraction, tissues were depolarized with potassium chloride (KCl, 60 mM) and subsequently washed with Krebs Henseleit solution. After 30 min, the aortic rings were precontracted by norepinephrine (NE, 3×10^{-7} M) and, when the contractile response was stabilized (steady-state phase at 12−15 min), their relaxation was evaluated by cumulative addition (from 10^{-10} to 10^{-4} M) of acetylcholine and *C. cardunculus* or apigenin and luteolin (as reference flavonoids). After washout and a further 30 min of equilibration, the aortic ring preparations were challenged by cumulative concentrations of endothelin-1 (ET-1, from 10^{-11} to 10^{-5} M) in the absence (vehicle) or presence of 1×10^{-4} M *C. cardunculus*, apigenin, or luteolin. Pretreatment with the compounds under investigation started 20 min before the dose−response curve with ET-1. Furthermore, at the end of each dose−response curve with ET-1, an aliquot (1−2 mL) of the medium was withdrawn from the incubation bath to quantify the amount of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin) released from the vascular tissue. The determination of 6-keto-$PGF_{1\alpha}$ was performed by a specific immunoassay kit, following the instructions of the manufacturer (Amersham Italia, Cologno Monzese, Milano, Italia), and the results were expressed as pg/mg of w.t.

Finally, after washout and when the tonus of the aortic rings had returned to baseline (about 40 min), the endothelial-dependent vasoconstriction was obtained by the addition of the nitric oxide synthase (NOS) inhibitor N^G-monomethyl-L-arginine (L-NMMA, 1×10^{-4} M), in the absence or presence of the compounds under investigations.

Effect of *C. cardunculus* Administration to Aged Rats. Male Fisher 344 × Norwegian Brown rats (500−600 g), aged 30 months (corresponding to a 65−75-year-old human), were obtained from Harlan (Correzzana, Milan, Italy). This is a well-characterized rat strain that is an approved rodent model for aging studies by the National Institutes of Health and Aging (NIH/NIA). Rats were housed under a standard light cycle and constant temperature. Water and food were provided at libitum. After 5 days, rats were placed in metabolic cages for the collection of 24-h urine. Then, the animals were randomly allocated into one of two groups: group 1 ($n = 6$), controls or group 2 ($n = 6$), which was daily-treated by oral gavage with the aqueous *C. cardunculus* extract (10 mg kg^{-1} day^{-1} of polyphenols), for 5 days. Control rats were given an equal amount of water. In addition, six young male Wistar rats (Charles River Laboratories, Calco, Italy), aged 8 months, were used to comparatively test the activity of wild artichoke and were daily administered water by gavage, for 5 days.

Isolated Rat Aortic Rings. At the end of the treatment period and 1 h after the last gavage, the rats were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.; Rhone Merieux, Pinkenba, Queensland, Australia) and the chests were opened to isolate the thoracic aorta, which were then cut, mounted, and processed as described above.

Blood Analyses. Before the isolation of aortic rings, blood was drawn from the heart and serum was separated by centrifugation at 14000*g* for 5 min and subsequently stored at −80 °C. Thromboxane B_2 (TXB_2) was quantified by immunoassay (Cayman Chemical, Ann Arbor, MI), following the instructions of the manufacturer. Total antioxidant capacity was quantified by a colorimetric assay (BIOXYTECH AOP-490, OxisResearch, Portland, OR), which is based on the reduction of Cu^{2+} to Cu^{+} (*13*). The results are shown as micromoles of Cu^{2+} reduced.

Urine Analyses. Before and after the treatments, 24-h urines were collected and centrifuged at 2800*g* for 10 min at 18 °C. The supernatant was stored at −80 °C. The F_2-isoprostane, namely, 8-iso-$PGF_{2\alpha}$, concentration in urine was quantified by immunoassay (Oxford Biomedical Research, Ann Arbor, MI), following the instructions of the manufacturer.

Statistical Analyses. The results are expressed as the mean ± standard error of the mean (SEM), and *n* indicates the number of experiments (rats). For each concentration−response curve, the maximum effect (E_{max}) and the concentration of agonist that produced one-half of E_{max} (EC_{50}) were estimated using nonlinear regression analysis (Prism version 2.0; GraphPad Software, Inc., San Diego, CA). The sensitivity of the agonist is expressed as pEC_{50}. One- and two-way analysis of variance (ANOVA) were followed by a Bonferroni *t* test, or paired or unpaired Student's *t* tests, as appropriate, were used for statistical analyses. A $p < 0.05$ was considered statistically significant.

Urinary and circulating markers were compared by unpaired Student's *t* test.

RESULTS

HPLC analysis of the wild artichoke extract confirmed its high proportion in phenolic compounds, in particular luteolin and apigenin, as previously shown by Llorach et al. (*14*) and us (*9*).

Direct Effect of *C. cardunculus* on Rat Aortic Rings. In this first set of experiments, the maximal contractions caused by NE in endothelium-intact aortic rings harvested from control rats were not significantly different in all groups (**Figure 1B**). The addition of graded concentrations of *C. cardunculus* extract or of its most important components (luteolin and apigenin) to NE-precontracted aortic rings led to a dose-dependent vasorelaxation (**Figure 1A**). This effect was not dependent upon the presence of the endothelium, because the dose−response curves obtained for all compounds were similar in vascular tissues when the endothelium was removed (**Table 1**).

As shown in **Figure 2A**, significantly improved vasomotion was observed when the aortic tissues were contracted by ET-1. In fact, the E_{max} obtained with ET-1 in vehicle-treated tissues (53 ± 5%) was significantly reduced in the presence of *C. cardunculus* (29 ± 2%; $p < 0.01$), luteolin (26 ± 3%; $p < 0.01$), or apigenin (40 ± 3%; $p < 0.05$). The concomitant release of 6-keto-$PGF_{1\alpha}$ because of ET-1 was significantly also increased by wild artichoke, to a degree similar to that of luteolin and apigenin (**Figure 2B**).

***Ex Vivo* Vasomodulating Effect of *C. cardunculus*.** In this set of experiments, the maximal contractions caused by NE in aortic rings prepared from vehicle-treated young and aged rats or from *C. cardunculus*-treated aged rats were not significantly different (**Figure 3A**). As compared with aged vehicle-treated rats (**Figure 3A**), treatment of aged animals with a *C. cardunculus* extract significantly increased acetylcholine-induced endothelial-dependent vasorelaxation in NE-precontracted aortic tissue ($E_{max} = 80 \pm 6$ versus 56 ± 4%; $p < 0.05$). However, the degree of this effect did not reach that observed in young animals treated with the vehicle ($E_{max} = 97 \pm 4\%$).

Data concerning the sensitivity of vascular smooth muscle to ET-1 and the simultaneous release of 6-keto-$PGF_{1\alpha}$ obtained

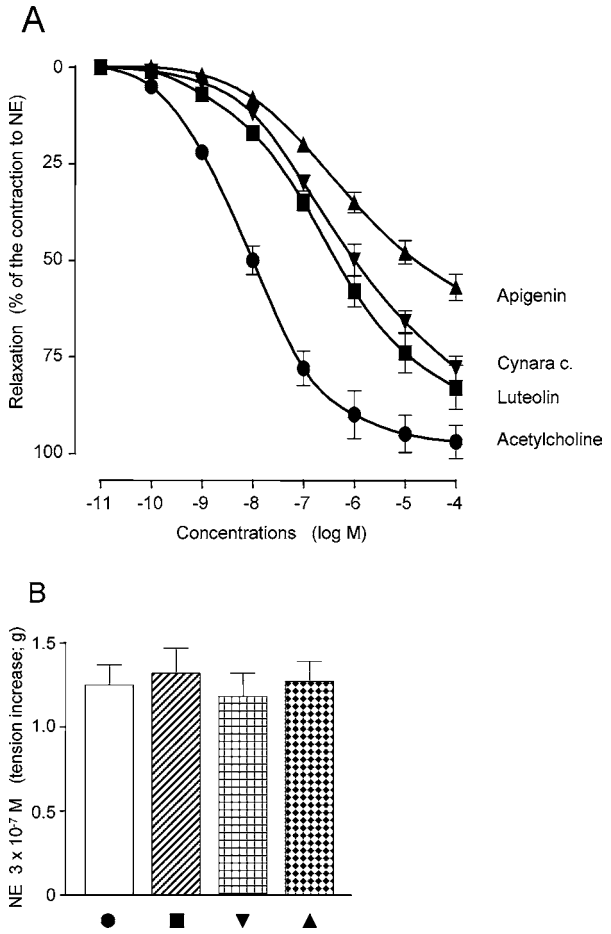

Figure 1. (A) Cumulative concentration–response curves to *C. cardunculus*, luteolin, apigenin, and acetylcholine in endothelium-intact rat aortic rings precontracted with NE (3×10^{-7} M). (B) Bar graphs show that, in the different groups, the vascular tissues were precontracted to a similar level with NE. All values are expressed as means ± SEM ($n = 6$). For a statistical comparison, see the values reported in **Table 1**.

Table 1. Dose–Response Parameters (pEC_{50} and E_{max}) for the Action of *C. cardunculus*, Leutolin, and Apigenin in Comparison to Acetylcholine in Endothelium-Intact or Endothelium-Denuded Rat Aortic Rings Precontracted with Norepinephrine[a]

	endothelium intact		endothelium denuded	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
C. cardunculus	6.59 ± 0.05^{b}	78 ± 5^{b}	6.41 ± 0.07	75 ± 6
luteolin	6.62 ± 0.06^{b}	83 ± 5^{b}	6.48 ± 0.11	87 ± 5
apigenin	$6.27 \pm 0.04^{c,d}$	$57 \pm 3^{c,d}$	5.91 ± 0.10^{c}	60 ± 5^{c}
acetylcholine	8.10 ± 0.06	97 ± 4	nd[e]	nd[e]

[a] Data, calculated from **Figure 1**, are means ± SEM ($n = 6$ each). [b] $p < 0.05$ versus acetylcholine. [c] $p < 0.05$ versus *C. cardunculus* and luteolin. [d] $p < 0.01$ versus acetylcholine. [e] nd = nondetectable.

in aortic rings harvested from different experimental groups of rats are reported in **Figure 4**. In aortic tissues from young vehicle-treated rats, the addition of cumulative concentrations of ET-1 increased the tension ($E_{max} = 53 \pm 4\%$) and prompted the simultaneous release of 6-keto-$PGF_1\alpha$ (295 ± 217 pg/mg of w.t.) into the medium. A conspicuous hyperactivity, with a marked reduction of 6-keto-$PGF_1\alpha$ generation, was observed when aortic rings from aged vehicle-treated rats were challenged with ET-1. In fact, the maximal vasoconstriction induced by ET-1 was $81 \pm 4\%$ ($p < 0.01$), and this phenomenon was paralleled by a decreased generation of 6-keto-$PGF_1\alpha$ ($182 \pm$

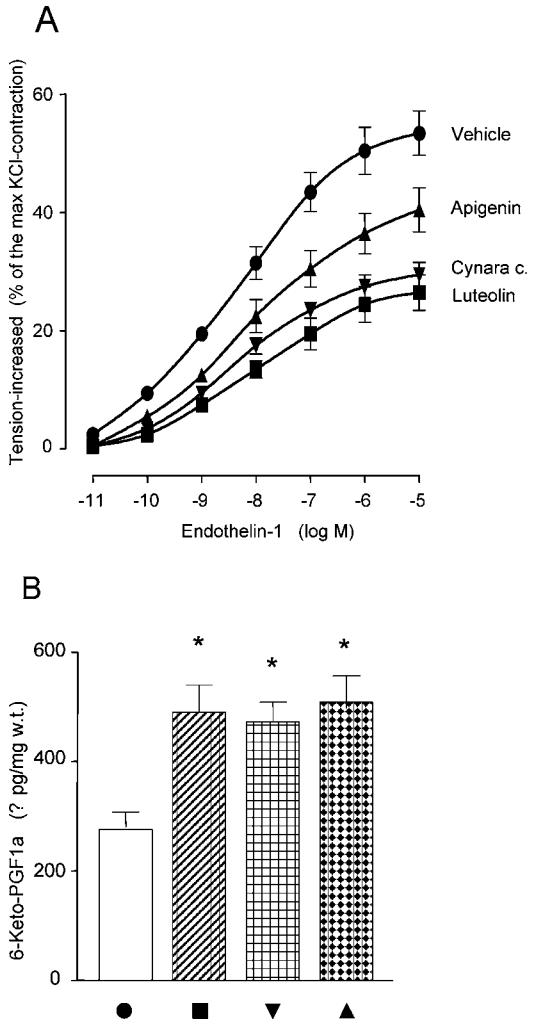

Figure 2. (A) Cumulative concentration–response curves to ET-1 in the presence of vehicle or *C. cardunculus*, luteolin, or apigenin (all at 10^{-4} M) in endothelium-intact rat aortic rings and (B) corresponding release of 6-keto-$PGF_1\alpha$ in these tissues. All values are expressed as means ± SEM ($n = 6$). Both *C. cardunculus* and luteolin ($p < 0.01$) are more potent that apigenin ($p < 0.05$) in reducing the vasoconstriction induced by ET-1 when compared to the vehicle.

13 pg/mg of w.t.; $p < 0.01$). Treatment of aged rats with *C. cardunculus* extract markedly reduced the hyperactivity of the aortic rings to ET-1 and was associated with a significant increase in the generation of the prostanoid (**Figure 5**).

The effects of *C. cardunculus* were in part dependent upon NO production, confirming *in vitro* data (*9*) and demonstrated by a significant reversal of the vasoconstrictive effect of L-NMMA (**Figure 5**). In fact, it is well-known that, in isolated vasculature, the increased tension that follows the inhibition of eNOS is an index of the integrity of endothelium-dependent vasomotion (*15*). Accordingly, when aortic rings from young vehicle-treated rats were challenged with L-NMMA, a progressive increase in tension was recorded, peaking at $27.8 \pm 2.4\%$. This event was markedly reduced in aortic preparations from aged vehicle-treated rats ($13.2 \pm 1.8\%$; $p < 0.01$). In contrast, in aortic rings from aged *C. cardunculus*-treated rats, the increase in tension in response to L-NMMA was almost completely restored (i.e., $22.4 \pm 1.6\%$; **Figure 5**).

***In Vivo* Effects of *C. cardunculus* on Surrogate Markers of CHD.** *Serum TXB_2.* Serum TXB_2 concentrations, a sensitive index of thromboxane production by fully activated platelets (*16*), were not affected by the treatment with *C. cardunculus.*

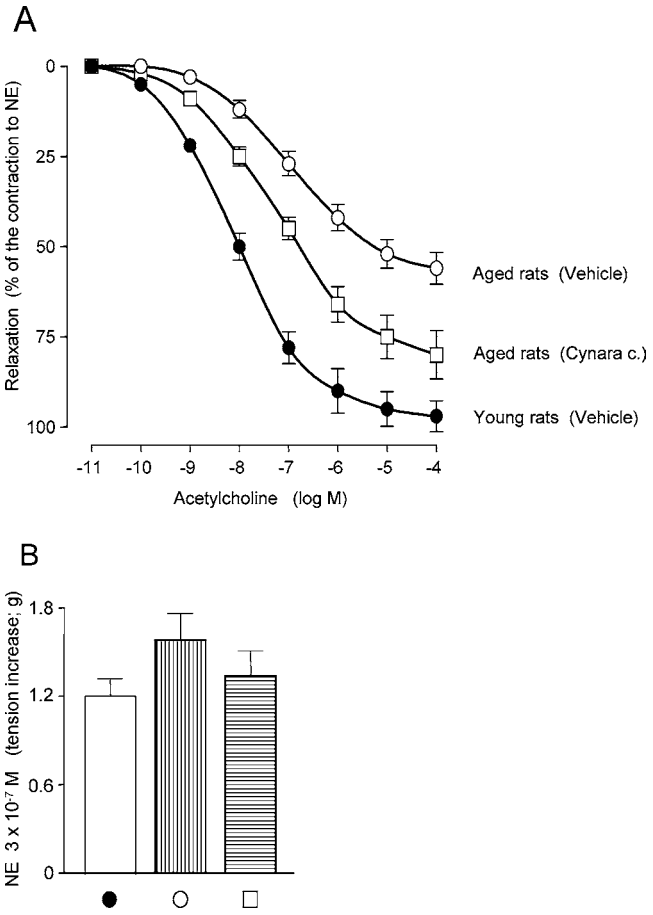

Figure 3. (A) Cumulative concentration–response curves of acetylcholine in NE-precontracted aortic rings prepared from vehicle-treated young and aged rats or from *C. cardunculus*-treated aged rats. (B) Bar graphs show that, in the three different groups, the vascular tissues were precontracted to a similar level with NE. All values are expressed as means ± SEM (n = 6 per group).

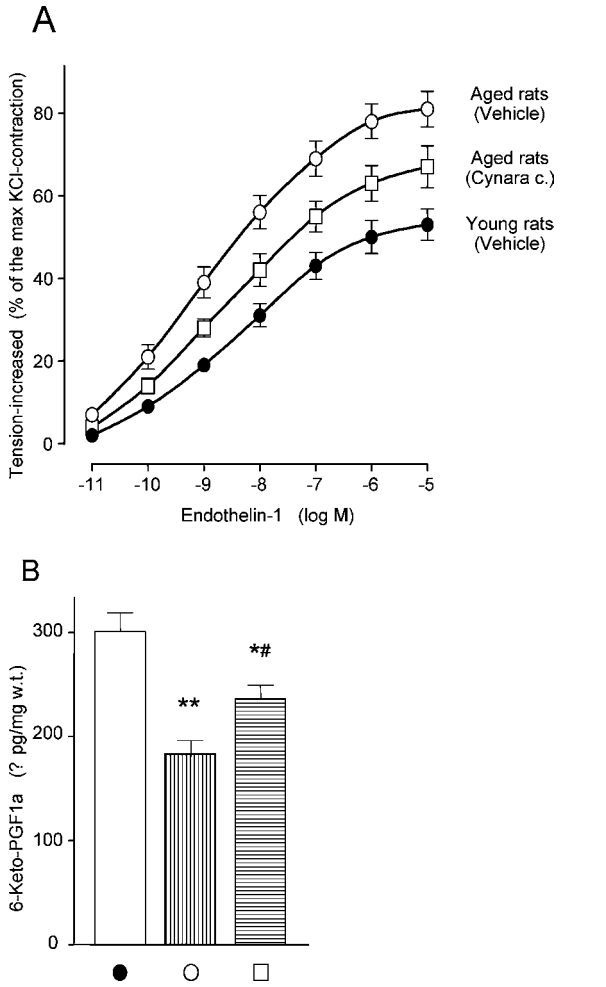

Figure 4. (A) Cumulative concentration–response curves of ET-1 in isolated aortic rings prepared from vehicle-treated young and aged rats or from *C. cardunculus*-treated aged rats; (B) concomitant 6-keto-$PGF_1\alpha$ release from these tissues. Points and columns are mean values ± SEM (n = 6 per group. (*) $p < 0.05$ and (**) $p < 0.01$ versus vehicle-treated young rats and (#) $p < 0.05$ versus vehicle-treated aged rats.

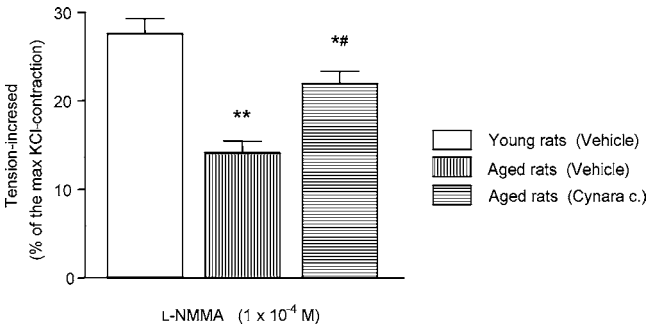

Figure 5. Increase in tension induced by nitric oxide synthase inhibition with L-NMMA in aortic rings prepared from vehicle-treated young and aged rats or from *C. cardunculus*-treated aged rats. Columns represent mean values ± SEM (n = 6). (*) $p < 0.05$ and (**) $p < 0.01$ versus vehicle-treated young rats and (#) $p < 0.05$ versus vehicle-treated aged rats.

In fact, after 5 days of extract administration by gavage, the concentrations in control rats were found to be 0.86 ± 1.45 μg/mL, while those in the treated rats were 0.87 ± 2.05 μg/mL (**Table 2**).

Serum Antioxidant Capacity. The total antioxidant capacity of serum was assessed by a colorimetric assay (*13*). As show in **Table 2**, the antioxidant capacity of the serum from control rats was 99.12 ± 4.24 μM Cu^{2+} reduced and that of the serum from treated rats was 110.42 ± 3.56 μM Cu^{2+} reduced. In turn, the treatment with an aqueous wild artichoke extract significantly increased serum antioxidant capacity by 11%.

Urinary 8-Iso-$PGF_{2\alpha}$ Excretion. The urinary concentrations of 8-iso-$PGF_{2\alpha}$ are reported in **Table 2**. The analyses of variance did not show any significant modification of excretion during the whole experimental period. In fact, 8-iso-$PGF_{2\alpha}$ excretion of the treated rats was 1.54 ± 2.05 pg/mL and that of the control rats was 1.55 ± 1.89 pg/mL.

DISCUSSION

In this study, we demonstrate that wild artichoke (*C. cardunculus*), a wild plant widely consumed in selected areas of the Mediterranean basin (*8*, *11*), ameliorates vasomotion in rat isolated aortic rings and partially but significantly restores proper vasoreactivity of the aged vessel after oral administration. This finding adds to the vast body of experimental research supporting the epidemiological evidence of a lower CHD incidence in the Mediterranean area. In particular, it suggests that the maintenance of correct vasomotion by bioactive components of the Mediterranean diet might contribute to its cardioprotective properties, as also experimentally confirmed in a cohort of hypertensive patients (*17*). However, most studies have thus far focused on the role of macronutrients, e.g., low intake of saturated fat, high intake of mono- and polyunsaturated

Table 2. Effects of *C. cardunculus* Treatment (10 mg kg^{-1} day^{-1} for 5 Days) of Aged Rats on Selected Surrogate Markers of Cardiovascular Disease[a]

parameter	control	treated
serum thromboxane B_2 (μg/mL)	0.86 ± 1.45	0.87 ± 2.05
serum antioxidant capacity (μM Cu^{2+} reduced)	99.12 ± 4.24	110.42 ± 3.56[b]
urinary 8-iso-$PGF_{2\alpha}$ (pg/mL)	1.55 ± 1.89	1.54 ± 2.05

[a] Data are means ± SEM ($n = 6$). [b] $p < 0.01$.

fat, and high intake of fiber and complex carbohydrates, in CHD prevention as assessed in the Mediterranean area. However, a role for oligonutrients is also rapidly emerging, mostly as a consequence of the oxidant/antioxidant theory of CHD (*18*). In fact, the mechanisms of endothelial dysfunction in vascular disease and aging are multifactorial; nevertheless, accumulating evidence suggests that oxidative stress is an important component (*19*, *20*). For example, oxidized lipids impair vascular reactivity and have been implicated in cardiovascular morbidity and mortality (*19*, *21*). Even though current data do not fully support a role for antioxidant therapy, namely, with vitamin E, in decreasing atherosclerosis, cardiovascular mortality (*22*, *23*), and more specifically endothelial dysfunction (*24*), clinical data indicate that vitamin C administration does improve vasorelaxation (*25*). Moreover, thiol agents such as lipoic acid and l-2-oxothiazolidine-4-carboxylic acid (OTC) have also been shown, *in vitro* (*26*, *27*) and *in vivo* (*28*), to increase endothelium-derived NO production. Possibly, water-soluble or amphiphilic antioxidants acting at the lipid−water interface effectively scavenge free radicals or modulate enzymatic processes relevant to cardiac and vascular functions, e.g., eNOS activity. This concept is corroborated by the finding (**Table 2**) that administration of wild artichoke to aged rats significantly increases serum antioxidant capacity, even though no effect was observed on isoprostane excretion. Possibly, micro-environmental modulation of redox events, together with the maintenance of proper cofactors, might take place and result in enhanced eNOS activity.

It is noteworthy that a stimulant effect was also noted with respect to the production of prostacyclin (**Figures 2** and **5**), whose pathways of production are independent from those of NO. This effect was noted both *in vitro* and after feeding of the extract. Thus, enhanced production of this vasorelaxant agent might add to that of NO and confer additional vasoprotective benefits to the elderly.

The observation, in isolated rat aortic rings, that vasorelaxation induced by wild artichoke and flavonoids, namely, apigenin and luteolin, was recorded even in the absence of the endothelium is consistent with previous *in vitro* data that indicated how the effects of *C. cardunculus* on the vessel were partially reversible by the addition of the eNOS inhibitor L-NMMA (*9*). This suggest a direct activity of *C. cardunculus* and its constituents on smooth muscles cells, additional to that on NO production (**Figure 5**), and through mechanisms that are still to be investigated.

Many other biological activities have also been demonstrated for *C. cardunculus*, including scavenging of free radicals (e.g., superoxide anion, hydroxyl radical, and hypochlorous acid) (*8*, *21*, *29*) and hepatoprotection (*14*). Finally, the effects of artichoke juice on the endothelial function of hyperlipidemic patients have been reported by Lupattelli et al. (*30*), who also demonstrated significant decreases in selected adhesion molecules, namely, ICAM-1 and VCAM-1.

In conclusion, this study provides further justification to the advice to consume wild greens as part of the diet and suggests that close attention should be paid to the diet of the elderly, because it can effectively modulate important parameters of cardiovascular risk.

LITERATURE CITED

(1) Tschudi, M. R.; Barton, M.; Bersinger, N. A.; Moreau, P.; Cosentino, F.; Noll, G.; Malinski, T.; Luscher, T. F. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. *J. Clin. Invest.* **1996**, *98*, 899−905.

(2) Frein, D.; Schildknecht, S.; Bachschmid, M.; Ullrich, V. Redox regulation: A new challenge for pharmacology. *Biochem. Pharmacol.* **2005**, *70*, 811−823.

(3) Schulz, E.; Anter, E.; Keaney, J. F., Jr. Oxidative stress, antioxidants, and endothelial function. *Curr. Med. Chem.* **2004**, *11*, 1093−1104.

(4) Hagen, T. M. Oxidative stress, redox imbalance, and the aging process. *Antioxid. Redox Signaling* **2003**, *5*, 503−506.

(5) Trichopoulou, A.; Critselis, E. Mediterranean diet and longevity. *Eur. J. Cancer Prev.* **2004**, *13*, 453−456.

(6) Visioli, F.; Borsani, L.; Galli, C. Diet and prevention of coronary heart disease: The potential role of phytochemicals. *Cardiovasc. Res.* **2000**, *47*, 419−425.

(7) Heinrich, M.; Leonti, M.; Nebel, S.; Peschel, W. "Local Food-Nutraceuticals": An example of a multidisciplinary research project on local knowledge. *J. Physiol. Pharmacol.* **2005**, *56* (supplement 1), 5−22.

(8) The Local Food-Nutraceuticals Consortium. Understanding local Mediterranean diets: A multidisciplinary pharmacological and ethnobotanical approach. *Pharmacol. Res.* **2005**, *52*, 353−366.

(9) Grande, S.; Bogani, P.; de Saizieu, A.; Schueler, G.; Galli, C.; Visioli, F. Vasomodulating potential of mediterranean wild plant extracts. *J. Agric. Food Chem.* **2004**, *52*, 5021−5026.

(10) Juhaszova, M.; Rabuel, C.; Zorov, D. B.; Lakatta, E. G.; Sollott, S. J. Protection in the aged heart: Preventing the heart-break of old age? *Cardiovasc. Res.* **2005**, *66*, 233−244.

(11) Pieroni, A.; Nebel, S.; Santoro, R. F.; Heinrich, M. Food for two seasons: Culinary uses of non-cultivated local vegetables and mushrooms in a south Italian village. *Int. J. Food Sci. Nutr.* **2005**, *56*, 245−272.

(12) Visioli, F.; Vinceri, F. F.; Galli, C. "Waste waters" from olive oil production are rich in natural antioxidants. *Experientia* **1995**, *51*, 32−34.

(13) Visioli, F.; Caruso, D.; Plasmati, E.; Patelli, R.; Mulinacci, N.; Romani, A.; Galli, G.; Galli, C. Hydroxytyrosol, as a component of olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Radical Res.* **2001**, *34*, 301−305.

(14) Llorach, R.; Espin, J. C.; Tomas-Barberan, 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.

(15) Feletou, M.; Vanhoutte, P. M. EDHF: New therapeutic targets? *Pharmacol. Res.* **2004**, *49*, 565−580.

(16) Patrono, C.; Ciabattoni, G.; Davi, G. Thromboxane biosynthesis in cardiovascular diseases. *Stroke* **1990**, *21*, IV130−IV133.

(17) Fuentes, F.; Lopez-Miranda, J.; Sanchez, E.; Sanchez, F.; Paez, J.; Paz-Rojas, E.; Marin, C.; Gomez, P.; Jimenez-Pereperez, J.; Ordovas, J. M.; Perez-Jimenez, F. Mediterranean and low-fat diets improve endothelial function in hypercholesterolemic men. *Ann. Intern. Med.* **2001**, *134*, 1115−1119.

(18) Visioli, F. Antioxidants in Mediterranean diets. *World Rev. Nutr. Diet.* **2000**, *87*, 43−55.

(19) Stocker, R.; Keaney, J. F., Jr. Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* **2004**, *84*, 1381−1478.

(20) Assanelli, D.; Bonanome, A.; Pezzini, A.; Albertini, F.; Maccalli, P.; Grassi, M.; Archetti, S.; Negrini, R.; Visioli, F. Folic acid and vitamin E supplementation effects on homocysteinemia, endothelial function, and plasma antioxidant capacity in young myocardial-infarction patients. *Pharmacol. Res.* **2004**, *49*, 79−84.

(21) Schaffer, S.; Eckert, G. P.; Muller, W. E.; Llorach, R.; Rivera, D.; Grande, S.; Galli, C.; Visioli, F. Hypochlorous acid scavenging properties of local Mediterranean plant foods. *Lipids* **2004**, *39*, 1239−1247.

(22) Kritharides, L.; Stocker, R. The use of antioxidant supplements in coronary heart disease. *Atherosclerosis* **2002**, *164*, 211−219.

(23) Visioli, F. The roller coaster of antioxidant therapy. *Curr. Med. Chem.* **2004**, *11*, 3.

(24) Visioli, F. Effects of vitamin E on the endothelium: equivocal? α-Tocopherol and endothelial dysfunction. *Cardiovasc. Res.* **2001**, *51*, 198−201.

(25) Carr, A.; Frei, B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Radical Biol. Med.* **2000**, *28*, 1806−1814.

(26) Huang, A.; Xiao, H.; Samii, J. M.; Vita, J. A.; Keaney, J. F., Jr. Contrasting effects of thiol-modulating agents on endothelial NO bioactivity. *Am. J. Physiol. Cell Physiol.* **2001**, *281*, C719−C725.

(27) Visioli, F.; Smith, A.; Zhang, W.; Keaney, J. F., Jr.; Hagen, T.; Frei, B. Lipoic acid and vitamin C potentiate nitric oxide synthesis in human aortic endothelial cells independently of cellular glutathione status. *Redox Rep.* **2002**, *7*, 223−227.

(28) Vita, J. A.; Frei, B.; Holbrook, M.; Gokce, N.; Leaf, C.; Keaney, J. F., Jr. L-2-Oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J. Clin. Invest.* **1998**, *101*, 1408−1414.

(29) Valentao, P.; Fernandes, E.; Carvalho, F.; Andrade, P. B.; Seabra, R. M.; Bastos, M. L. Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. *J. Agric. Food Chem.* **2002**, *50*, 4989−4993.

(30) Lupattelli, G.; Marchesi, S.; Lombardini, R.; Roscini, A. R.; Trinca, F.; Gemelli, F.; Vaudo, G.; Mannarino, E. Artichoke juice improves endothelial function in hyperlipemia. *Life Sci.* **2004**, *76*, 775−782.

Received for review October 10, 2005. Revised manuscript received November 9, 2005. Accepted November 9, 2005. This work was supported by EEC Grant QLK-2001-00173 (Local Food Nutraceuticals).

JF052499S