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Protection of Lipids from Oxidation by Epicatechin, *trans*-Resveratrol, and Gallic and Caffeic Acids in Intestinal Model Systems

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Consumption of polyphenols is associated with health promotion through diet, although many are poorly absorbed in animals and humans alike. Lipid peroxides may reach the intestine and initiate deleterious oxidation. Here we measured inhibition of the oxidation of linoleic acid (LA) in authentic fluid from rat small intestine (RIF) by two dietary polyphenols, a flavonoid, epicatechin (EC), and a stilbene, resveratrol (RV), and by gallic (GA) and caffeic (CA) acids, and their partition coefficients. Both polyphenols inhibited 80%, and CA inhibited 65%, of the production of hexanal. GA was the weakest antioxidant in this assay. Interestingly, measuring peroxides production in RIF showed that only epicatechin inhibited the first stage of oxidation. The oxidizing agent, the antioxidant comound, the solution pH and lipophilicity are known to affect the total antioxidative activity. We suggest that the mechanism of this activity changes in accord with the environment: i.e., RV may act as a free radial scavenger, but here, in protecting lipids in intestinal fluid from oxidation, it acts as a hydrogen atom donor. Since the concentration of phenolics is much higher in the intestinal fluid than is ever achieved in plasma or other body tissues, it is suggested that their antioxidant activity could be exerted in the gastrointestinal tract (GIT), breaking the propagation of lipid peroxides oxidation and production of toxic compounds.

KEYWORDS: Gastrointestinal tract; dietary antioxidants; phenolics; partition coefficient

INTRODUCTION

It is widely recognized that diets rich in plant foods, such as traditional Mediterranean diets, promote health. This beneficial effect is associated with a lower risk of developing cardiovascular disease and certain cancers (*1*−*5*). Fruits, vegetables, breads, nuts, seeds, wine, and olive oil are all characteristic ingredients of the Mediterranean diets (*6*, *7*). Consumption of food and beverages rich in polyphenols, such as red wine and tea, has also drawn interest due to the positive correlation between consumption and prevention of disease (*1*, *7*, *8*). The wide range of biological effects exhibited by polyphenols is commonly suggested to be due to their powerful antioxidant properties in the body together with their ability to interact with redox-sensitive cell-signaling pathways (*5*, *8*, *9*).

It is usually recognized that the extent of the potency of polyphenols in vivo is dependent upon their absorption, metabolism, and secretion (*1*, *2*, *3*, *5*). Some, but not all, polyphenols can be absorbed through the gastrointestinal tract (GIT), but their uptake is incomplete and their plasma and body levels are low (*1*−*4*). Indeed, Halliwell and co-workers in pioneering works pointed to and demonstrated the presence of intact polyphenolics and suggested their importance as antioxidants in exerting health-promoting activities in the GIT (*4*, *9*, *10*). A few other works support the mode of beneficial action in the GIT, i.e., suggesting the stomach to function as an oxidizing bioreactor (*11*, *12*), or affecting deleterious microbial populations in the GIT (*13*, *14*).

Dietary lipids compose a major target for deleterious oxidation through both food processing and digestion (*15*, *16*). Reactions between free radicals generated during digestion and other food constituents dramatically alter the range of carcinogenic, cytotoxic, and atherogenic compounds produced; these include lipid hydroperoxides, oxycholesterols, malondialdehyde, and hydroxy alkanals (*17*). Recent studies have demonstrated that oxidized fatty acids in the diet result in oxidized lipids in serum lipoproteins, in rodents. In rats and humans the level of oxidized fatty acids in the serum are proportional to the quantity of oxidized fatty acids in the diet, with increased dietary oxidized fatty acids leading to increased exogenous and endogenous

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serum oxidized lipoprotein levels (*18*, *19*). Others also demonstrated that dietary lipid oxidation products are absorbed in man (*20*, *21*).

Dietary antioxidants, including plant phenolics, may be of major importance in inhibiting lipid oxidation at those sites where their levels are highest and adverse oxidation occurs, such as in the GIT (*4*, *10*). Here we studied the protection of lipids from deleterious oxidation, measured as inhibition of peroxide production and the consequent oxidative cleavage of LA by two dietary polyphenols, epicatechin (EC) and *trans*-resveratrol (RV), and by gallic (GA) and caffeic (CA) acids, in authentic fluid from rat small intestine (RIF) and in model solutions.

MATERIALS AND METHODS

Materials. Resveratrol (RV), epicatechin (EC), caffeic acid (CA), gallic acid (GA), ethylenediamine tetra acetic acid (EDTA), α-keto-γ-methiolbutyric acid (KMBA), 1-octanol, water-soluble LA, potassium hydrophosphate, potassium iodide, sodium azide, igepal, alkylbenzyldimethyl ammonium chloride, ammonium molybdate, and 2,2′-azobis(2-methylepropionamidine) dihydrochloride (AMPH) were purchased from Sigma (St. Louis, MO). Sodium acetate, acetic acid, sodium phosphate, and sodium phosphate 7-hydrate were purchased from Baker J. T. (Phillipsburg, NJ). Iron sulfate 7-hydrate and sodium chloride were purchased from BDH (Poole, UK). Hydrogen peroxide solution (30%) was purchased from Merck (Darmstadt, Germany). Fresh rat intestinal fluids (RIF) were collected from the jejunum of male Sprague Dawely rats (450 g body weight).

Antioxidant Activity of Polyphenols in Rat Intestinal Fluids (RIF). LA (2.7 mM) and AMPH (1 mM) were added ex vivo to RIF. Polyphenol (100 μL of a 10 mM solution) was added to 900 μL of RIF and the mixture was mixed for 120 min at 37 °C. After 90 and 120 min, 300 μL was taken from the reaction, added to 100 μL of methanol, and centrifuged (2 min at 14000*g*). The polyphenol content in the suspension was measured by HPLC and compared to the initial concentration (1 mM). The HPLC system (Thermo-Spectra) consisted of an autosampler (AS3000), injector (100 μL) column oven (*30*), pump (P3000), and a diode array detector (UV6000). A reversed-phase C-18 column (25 × 4.6 mm, Goldsil) was employed. Elution was performed using water and methanol, acidified with 0.01% (v/v) formic acid, at a flow rate of 1 mL/min. The entire experiment was repeated three times.

Analysis of Hexanal in Headspace. The procedures used were adapted from Frankel et al. (*22*, *23*) and Sanches-Silva et al. (*24*). LA was added as above, and phenolics were added to a final concentration of 1 mM. The samples were oxidized by adding myoglobin to a final concentration of 20 μM and stirring at 37 °C in a sealed amber vial. Aliquot samples (500 μL) were taken for hexanal analysis after 3 h. Hexanal was measured by static headspace gas chromatography (GC) as described previously (*24*). The headspace volatiles were collected by SPME, which consisted of the following sorptive coating materials: divinylbenzene, carboxen, and polydimethylsiloxane (2 cm −50/30 μm DVB/CAR/PDMS StableFlex, Supelco, Bellefonte, PA). Sampling was started by insertion of SPME fiber through the septum of a 4 mL vial into the headspace of an incubated and agitated sample. The sampling temperature and duration were 37 °C and 20 min, respectively.

Volatiles were desorbed at the injector port of the GC for 3 min in a splitless mode. The GC injector port was operated at 230 °C. GC analysis was performed using Hewlett-Packard series 6890 GC (Palo Alto, CA), injector equipped with a flame ionization detector (FID), and DB-WAX capillary column (60 m × 0.32 mm internal diameter, 0.25 μm film; J&W Scientific Co., Folsom, CA). The temperature program was as follows: 40 °C for 4 min, then raised constantly at 5 °C min^{-1} until 110 °C, and then raised again constantly at 90 °C min^{-1} until 200 °C where the temperature was maintained for 2 min. N2, the carrier gas, was supplied at a flow rate of 20 mL min^{-1}. FID was operated at 250 °C.

Lipid Peroxides in RIF. The procedure used was modified from el-Saadani et al. (*25*): Briefly, a sample of 100 μL was taken from the above reaction after 120 min and the peroxide value was measured by a spectrophotometric assay.

Total Oxygen Scavenging Capacity (TOSC) Assay (*26*). *1. AMPH as Radical Generator.* Peroxyl free radicals were generated by thermal homolysis of AMPH at 40 °C. Fresh stock solutions were prepared daily, containing 10 mM AMPH in potassium phosphate buffer pH 7, 100 mM potassium phosphate buffer pH 7.4, sodium acetate buffer pH 3, and 10 mM KMBA. RV, CA, EC, and GA were added to the reaction mixtures to a final concentration of 1 mM and ethylene was measured in headspace to determine TOSC values (each in five replicates). Reactions were carried out in 21 mL rubber-septum-sealed vials in a final volume of 2.1 mL. KMBA (200 μL) was added to 650 μL of buffer (50 mM phosphate at pH 7.4) and 1 mL of antioxidant solution. The reactions were initiated by injection of 240 μL of AMPH. Ethylene production was measured by gas chromatography analysis of 2 mL aliquots taken directly from the headspace of the reaction vial after 2 h. Analyses were performed with a Varian 3300 GC equipped with a flame ionization detector (FID), D-2500, chromato-integrator (Merck Hitachi, Darmstadt, Germany).

2. Ferrous Ion as a Radical Generator. Hydroxyl free radicals were generated by ferrous ion in a slight basic solution (intestinal pH). Fresh stock solutions were prepared daily, containing 10 mM Fe^{2+} in 20 mM EDTA, 10 mM H_2O_2, 100 mM potassium phosphate buffer pH 7.4, and 10 mM KMBA. The TOSC values were as above using the afore-listed phenolics at 1 mM (each in five replicates) in the buffer solutions. Reactions were carried out in 21 mL rubber-septum-sealed vials in a final volume of 2.1 mL. KMBA (200 μL) was added to 530 μL of buffer (phosphate or acetate) and 1 mL of antioxidant solution. The reactions were initiated by injection of 240 μL of Fe^{2+} solution and 120 μL of H_2O_2. Ethylene production was measured by gas chromatography analysis of 2 mL aliquots taken directly from the headspace of the reaction vial after 15 min. Analyses were performed as already described.

Partition Coefficient. Partition coefficients were determined according to Privat et al. (*27*). Equal volumes of buffer (50 mM phosphate at pH 7.4) and 1-octanol were shaken together and the layers were separated by centrifugation for 10 min at 1500*g*. Phenolics (4 mM) were dissolved in 5 mL of each buffer-saturated 1-octanol and then 1 mL from the latter was mixed with 1 mL of the matching buffer solution (in four replicates). The mixes were shaken for 1 h at 37 °C and then separated by centrifugation. The amount of the compounds in each fraction was measured by HPLC. The partition coefficient was the ratio of the area under the curve (AUC) of 1-octanol solutes to the area of the buffer solution. Similar experiments were performed with saline buffers, containing 0.9% NaCl. The entire experiment was repeated three times.

Statistical Analysis. Statistical significance between samples was calculated using the Turkey-Kramer HSD test employing JMP 3.1 software (SAS Institute, Cary, NC).

RESULTS

Headspace gas chromatography was used to measure hexanal production as a marker of LA oxidation (**Figure 1**). The antioxidant capacities of two polyphenols, EC and RV, and the phenolic acids CA and GA were evaluated and compared in an authentic RIF. Both EC and RV inhibited 85% of hexanal production as measured by GC/MS-SPME. Adding CA to the reaction mixture resulted in a substantial inhibition (65%) of production of hexanal from LA as compared to the reference reaction, but GA prevented only 15% of the production of hexanal.

Peroxide value served to evaluate the extent of the initial oxidation steps of LA in RIF in the above reactions, by the selected phenolics (**Figure 2**). The oxidation of phenolics was also determined in this system using reversed phase chromatography to measure their residual levels and expressed as millimoles using standard curves. EC was found to be the most potent in inhibiting accumulation of peroxides, using AMPH

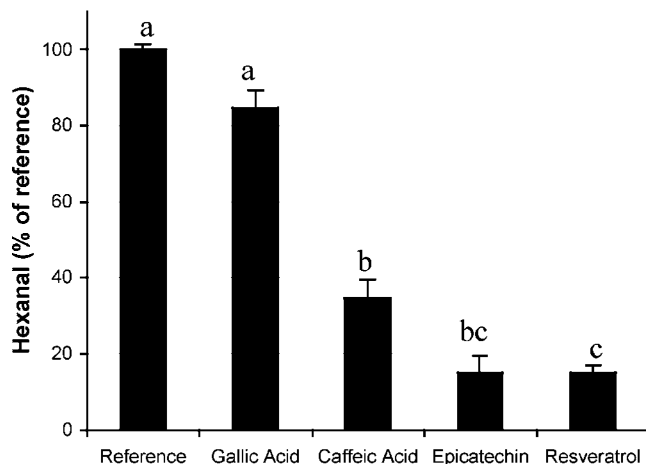


Figure 1. Hexanal in headspace of RIF after 3 h reaction, in the presence of phenolics (1 mM), expressed as percentage of hexanal produced in a reference reaction containing 2.7 mM LA and 20 μM myoglobin. Statistical comparison for all pairs was made using Student's T-test. Means designated by the same letter are not significantly different at $P = 0.05$. Bars represent standard deviation.

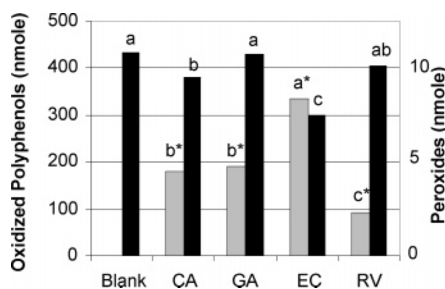


Figure 2. Oxidized polyphenols (gray bars) and peroxides (black bars) in the intestinal fluid after 3 h reaction. Statistical comparison for all pairs was made using Turkey-Kramer HSD. Means designated by the same letter are not significantly different at $P = 0.05$.

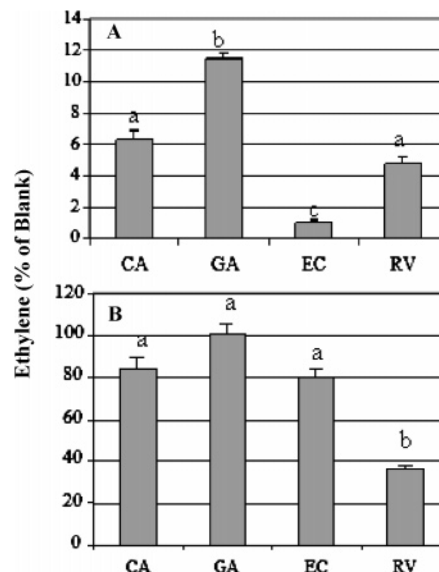


Figure 3. Inhibition of ethylene production by polyphenols, using AMPH (**A**) or ferrous ion (**B**) to generate free radicals at pH = 7.4. Statistical comparison for all pairs was made using Student's T-test. Means designated by the same letter are not significantly different at $P = 0.05$. Bars represent standard deviation.

Table 1. Comparison of the Partition Factor (log of Concentration) between Octanol and Dilute Buffer or Saline Buffer of Each Selected Polyphenol (Using 4 μmol)

	partition at pH 7.4 (log)	
	buffer	saline buffer
RV	1.87	2.79
EC	0.072	0.146
GA	−0.55	−1.155
CA	−1.03	−0.4

(1 mM) as an oxidizing agent in a 1 mL reaction mixture. The level of accumulated lipid peroxides produced from LA in RIF was 70% as compared to that in the reference reaction. This activity also resulted in the reduction of 333 nmol of the original EC. Inhibition of 15% with the reduction of 190 nmol was afforded by CA. A reduction of 190 nmol was also found for GA but the level of peroxides was similar to the reference reaction. Reduction in the level of RV in RIF was the lowest (90 nmol), resulting in only 9% inhibition of peroxide accumulation. It may be concluded here that the inhibition of the first steps of autoxidation of LA, i.e., production of peroxides, results in a proportional loss of RV, CA, and EC—higher activity resulted in higher decrease of the antioxidant. However, in the case of GA, a loss of 20% of the parent phenolic during the assay did not inhibit the production of lipid peroxides.

To extend our understanding of the rate of oxidation events, we have used the TOSC assay to measure antioxidant activity in in vitro model solutions and two water-soluble systems to generate the free radicals (*28*). With use of AMPH as the radical generator (**Figure 3A**), it was shown that with an intestinal pH (7.4) model solution, which also resembles plasma pH, the four selected phenols blocked almost the entire formation of ethylene from KMBA with RV and EC being the better antioxidants. When ferrous ion was used to generate free radicals (**Figure 3B**), only RV potently inhibited of the KMBA oxidation, demonstrating 64% inhibition. Both EC and CA inhibited only 20% of KMBA oxidation, while GA showed no antioxidant capacity at all.

The octanol−water partition coefficient is the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature. Octanol is an organic solvent that is used as a surrogate for natural lipids. It was suggested to affect oxidation rates (*27*, *29*). Here, we measured and compared the partition of the selected polyphenols in an octanol−saline buffer system to the commonly used octanol−water at intestinal pH (**Table 1**). Both GA and CA showed better solubility in buffer than in octanol at pH 7.4 ((log concentration in octanol/concentration in water) < 0). However, the opposite effect was detected when measuring the equilibrium concentration in an octanol−saline system: the concentration of GA in octanol has decreased, while the amount of CA dissolved in octanol has increased. When the partition of flavonoid or hydroxystilbene is assayed, the use of saline resulted in a much higher coefficient—these polyphenols were displaced into the octanol. Generally, EC is hydrophilic in nature; thus, its partition coefficient values are close to those of the phenolic acids. RV (a hydroxystilbene) has the higher partition coefficient in this work.

It is noteworthy that a rapid polymerization of polyphenols was observed in buffer at pH 7.4. To avoid this polymerization, and to ensure that we were measuring the intact polyphenol in each phase, we modified the common procedure. We found that with use of a vortex and centrifuge we could get valid partition coefficients in a short time, in accordance with other reports

(*27*). Using the area under the curve of HPLC chromatograms (rather than spectrophotometric determinations), we were able to measure the concentrations of the intact polyphenol in both phases. We found the overall recovery to be above 90% in all cases.

DISCUSSION

The level of hexanal, an end product of lipid oxidation, in headspace has previously been used as an indicator of lipid oxidation (*22*, *24*, *30*). It has also served to evaluate antioxidant capacity, especially in multicomponent systems, where antioxidants may fortify each other by cooperative effects, resulting in an increase of antioxidant activities (*30*). Here we found that two dietary polyphenols, EC and RV, were very potent in inhibiting hexanal production from linoleic acid added to authentic RIF. Dietary lipids reaching the upper intestine have already been exposed to oxidation in earlier stages, as early as food processing; thus, some are at the form of lipid peroxides (*12*). These lipid peroxides can serve as oxidizing agents, leading to propagation of the oxidation chain reaction, and the production of toxic compounds. Our results demonstrate a high potency of polyphenols in blocking the oxidation reaction as lipid peroxides enter the intestinal environment, and protect the body from deleterious actions of the lipid oxidation products.

CA was less potent than the tested polyphenols, and GA had a negligible, but not pro-oxidant activity, in contrast to other reports suggesting CA to be a poor antioxidant and GA to be a pro-oxidant. These results demonstrate the uniqueness of antioxidant activity of phenolics in the complex intestinal medium, and the need for understanding the mechanism of intestinal antioxidant activity of phenolics.

Lipids are enzymatically hydrolyzed in the upper intestine yielding free fatty acids, and phenolics are at their highest concentration at this same location (*10*). The levels of phenolics in the upper intestine may reach a local concentration of 3 mM, and we have thus used 1 mM concentrations to evaluate the antioxidant activity. In recent key reports, the contents of the intestine, and more specifically its aqueous phase, were suggested to hold most dietary phenolics that are consumed (*9*).

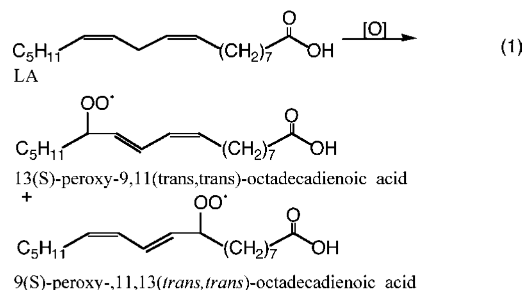
To better understand the individual contribution of each compound to the protection of lipids demonstrated here in RIF, we have designed model solutions: The antioxidant capacity of the four phenolics was tested at the intestinal pH, against hydroxyl radicals (generated through ferrous ion) and alkyl peroxy radicals (generated through the oxidation of an azo compound). Both radical-generating systems are hydrophilic in nature (*28*). We found no correlation of the activity measured as hexanal production in RIF with the activity measured in the model solutions: Although each phenolic had a different antioxidant activity, all four phenolics were very potent in blocking oxidation by alkyl peroxy radicals in buffer. However, measuring antioxidant activity against hydroxyl radicals varied greatly, suggesting that this assay is more informative: RV was best and EC second when a modified Fenton system (ferrous ion at pH = 7.4) was used but the phenolic acids showed little antioxidant (CA) to pro-oxidant (GA) activities. The pro-oxidant activity of GA in model solutions was demonstrated many times (*30*, *31*).

Different mechanisms were suggested to explain the action of phenolic antioxidants, including free radical scavenging activity, metal chelation, protein binding (*30*), and complex interfacial phenomena in heterogeneous systems where hydrophilic phenolic compounds vary in their partition behaviors between the water and oil phases and their interface (*30*, *31*). Intestinal contents are characterized as being very heterogeneous, and in an attempt to simplify the measurement we found that while using TOSC and AMPH in buffer does not provide meaningful data, using ferrous iron at intestinal pH did prove useful in indicating the potential of a tested compound to protect against lipid oxidation in the intestine. Still, RV seemed to be more potent than EC in this assay, in contrast to the results obtained in RIF. We hypothesized that these differences may be partly due to the great variability in the partition coefficients of the studied phenolics.

Commonly, the partition coefficient of a compound is defined as the ratio of its solubility in octanol to its solubility in water. This ratio may largely affect the potency of a compound to protect lipids from oxidation. At intestinal pH, both phenolic acids were more soluble in buffer than in octanol; GA appeared more lipophilic, and thus it was expected to act stronger in inhibiting oxidation. With this in mind, we used saline buffer and found that salinity has a major effect on the partition coefficients of all four phenolics. The solubility of GA in octanol decreased with the addition of sodium chloride, while those of CA, RV, and EC in octanol, like that of most of aromatic substances, increase with the addition of salt. Previous studies using water and octanol generalized that, in the case of protecting LDL, lipophilic antioxidants usually demonstrate more potent scavenging activity (*18*, *27*, *30*). When the data are used as part of the characterization of biological systems, we suggest that saline buffer is the appropriate aqueous model solution.

Partition coefficients did not provide a complete explanation for inhibition of the oxidation of linoleic acid in RIF. To evaluate the actual protection of fatty acids in the intestine, afforded by phenolics, we measured the accumulation of lipid peroxides, and its accompanying decrease in the level of phenolics. We again used AMPH since it continuously produces alkyl peroxy radicals at 37 °C, this time in intestinal fluid, as a simulation of oxidation processes taking place in the intestine. The fluid pH was 7.4; thus, the antioxidant protection afforded by EC and RV was expected to be the highest. Again, EC was indeed the better antioxidant, but RV failed to inhibit peroxide production from linoleic acid. Moreover, after 120 min in the RIF, only 10% of the original RV was degraded, leaving more intact RV than any of the other phenolics.

It is well-recognized that consistently with the classical free radical mechanism of autoxidation, involving a pentadiene intermediate having equivalent sites for oxygen attack at carbon-9 and carbon-13, equal amounts of 13- and 9-peroxides (see reaction (1)) are the major products of autoxidation (1) of linoleic acid at different temperatures and peroxide levels (*30*).



Usually, the breakdown of lipid hydroperoxides to free radicals is catalyzed in the presence of myoglobin, enhancing the propagation step and general peroxidation as demonstrated and discussed previously (*11*, *12*, *32*). The protonated form of ferryl myoglobin ($Fe^{4+}-OH^-$) was suggested to be the reactive

species regulating the peroxidative activity of myoglobin (*33*), abstracting an electron from either the protein or porphyrin, and allowing fast regeneration of the ferric species. However, alkaline conditions, as those prevalent in the intestine, stabilize the ferryl species, making myoglobin considerably less reactive toward lipids and lipid hydroperoxides (*33*). Based on the latter, we suggest that while EC inhibits the autoxidation of LA, RV in RIF acts as a hydrogen atom donor (*2*), leading to accumulation of lipid hydroperoxides and thus preventing the propagation step and the co-oxidation and formation of hexanal. Such a mechanism was suggested for the antioxidant activity of tocopherol (*4*, *31*).

$$\mathrm{RV{-}OH + LOO^{\bullet} \rightarrow RVO^{\bullet} + LOOH} \quad (2)$$

Our results demonstrate the need for an accepted valid assay for antioxidant activity in lipophilic systems (*1*, *5*, *8*, *29*, *31*). It is suggested that the TOSC assay using ferrous radical to initiate oxidation in aqueous solution correlates well with the production of hexanal from linoleic acid in the complex reaction in RIF. Due to the high relevance of phenolics in the small intestine in providing antioxidant protection before lipids are absorbed (*2*, *4*, *9*, *10*, *12*), we find the hexanal production accompanied by peroxide value as the most important and credible assays.

Preventing degenerative diseases through dietary intervention has enormous potential as a simple, widely acceptable, and inexpensive way of improving human health (*2*−*6*, *9*, *11*, *34*). As data are starting to accumulate on the absorption of oxidized fatty acids, lipids cholesterol, and lipoproteins from the intestine and on the action of free radicals in the intestine (*15*, *17*−*19*), our results extend and support a new approach suggested by Halliwell and colleagues (*4*, *9*, *10*) and by Kanner and Lapidot (*12*) for the health benefits of the active but poorly absorbed plant polyphenols: Regular consumption of dietary antioxidants may dramatically reduce deleterious oxidative processes in the GI tract. A detailed study of the distribution of free radicals and antioxidants, and their activities in the GI tract that include antioxidant and anticancer activities (*4*, *6*, *9*−*12*, *14*), will lead to the establishment of how ingested antioxidants can afford protection through diet. In addition, this information may be utilized to tailor site-specific antioxidants with superior activities. The potential for developing chemo-preventive diets that will improve human health is extremely attractive.

ABBREVIATIONS USED

AMPH, 2,2′-azobis(2-methylepropionamidine) dihydrochloride; CA, caffeic acid; EC, epicatechin; GA, gallic acid; GIT, gastrointestinal tract; KMBA, α-keto-γ-methiolbutyric acid; LA, linoleic acid; LDL, low-density lipoprotein; RIF, rat intestinal fluids; RV, resveratrol; TOSC, total oxygen scavenging capacity.

LITERATURE CITED

(1) Serafini, M.; Del Rio, D.; Crozier, A.; Benzie, I. F. F. Effect of changes in fruit and vegetable intake on plasma antioxidant defenses in humans. *Am. J. Clin. Nutr.* **2005**, *81*, 531−532.

(2) Rice-Evans, C. Flavonoids and isoflavones: Absorption, metabolism, and bioactivity. *Free Radical Biol. Med.* **2004**, *36*, 827−828.

(3) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230s−242s.

(4) Halliwell, B.; Rafter, J.; Jenner, A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *Am. J. Clin. Nutr.* **2005**, *81*, 268s−276s.

(5) Serafini, M.; Del Rio, D. Understanding the association between dietary antioxidants, redox status and disease: is the Total Antioxidant Capacity the right tool? *Redox Rep.* **2004**, *9*, 145−152.

(6) Pitsavos, C.; Panagiotakos, D. B.; Tzima, N.; Chrysohoou, C.; Economou, M.; Zampelas, A.; Stefanadis, C. Adherence to the Mediterranean diet is associated with total antioxidant capacity in healthy adults: the ATTICA study. *Am. J. Clin. Nutr.* **2005**, *82*, 694−699.

(7) Heinrich, M.; Leonti, M.; Nebel, S.; Peschel, W.; Pieroni, A.; Smith, F.; Rivera, D.; Obon, C.; Inocencio, C.; Verde, A.; Fajardo, J.; Llorach, R.; Muller, W. E.; Eckert, G. P.; Schaffer, S.; Schmitt-Schillig, S.; Antonopoulou, S.; Kypriotakis, Z.; Manios, Y.; Nomikos, T.; Kaliora, A.; Sidossis, L.; Galli, C.; Visioli, F.; Grande, S.; Bogani, P.; de Saizieu, A.; Fluhmann, B.; D'Orazio, D.; Fowler, A.; Koj, A.; Bereta, J.; Dulak, J.; Guzdek, A.; Kapiszewska, M. Understanding local Mediterranean diets: A multidisciplinary pharmacological and ethnobotanical approach. *Pharmacol. Res.* **2005**, *52*, 353−366.

(8) Pellegrini, N.; Serafini, M.; Colombi, B.; Del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J. Nutr.* **2003**, *133*, 2812−2819.

(9) Jenner, A. M.; Rafter, J.; Halliwell, B. Human fecal water content of phenolics: The extent of colonic exposure to aromatic compounds. *Free Radical Biol. Med.* **2005**, *38*, 763−772.

(10) Halliwell, B.; Zhao, K. C.; Whiteman, M. The gastrointestinal tract: A major site of antioxidant action? *Free Radical Res.* **2000**, *33*, 819−830.

(11) Lapidot, T.; Granit, R.; Kanner, J. Lipid hydroperoxidase activity of myoglobin and phenolic antioxidants in simulated gastric fluid. *J. Agric. Food Chem.* **2005**, *53*, 3391−3396.

(12) Kanner, J.; Lapidot, T. The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biol. Med.* **2001**, *31*, 1388−1395.

(13) Puupponen-Pimia, R.; Nohynek, L.; Hartmann-Schmidlin, S.; Kahkonen, M.; Heinonen, M.; Maatta-Riihinen, K.; Oksman-Caldentey, K. M. Berry phenolics selectively inhibit the growth of intestinal pathogens. *J. Appl. Microbiol.* **2005**, *98*, 991−1000.

(14) Kerem, Z. On the fate of herbal medicines and natural products in the gastrointestinal tract. In *Handbook of medicinal plants*; Yaniv, Z., Bacharach, U., Eds.; Haworth Press Inc.: New York, 2004; pp 429−444.

(15) Staprans, I.; Rapp, J. H.; Pan, X. M.; Feingold, K. R. Oxidized lipids in the diet are incorporated by the liver into very low density lipoprotein in rats. *J. Lipid Res.* **1996**, *37*, 420−430.

(16) Dobarganes, C.; Marquez-Ruiz, G. Oxidized fats in foods. *Curr. Opin. Clin. Nutr. Metabol. Care* **2003**, *6*, 157−163.

(17) Staprans, I.; Pan, X. M.; Rapp, J. H.; Feingold, K. R.; Kubow, S. Oxidized cholesterol in the diet accelerates the development of aortic atherosclerosis in cholesterol-fed rabbits: Routes of formation and toxic consequences of lipid oxidation products in foods. *Arterioscler., Thromb., Vasc. Biol.* **1998**, *18*, 977−983.

(18) Staprans, I.; Pan, X. M.; Rapp, J. H.; Feingold, K. R. Oxidized cholesterol in the diet is a source of oxidized lipoproteins in human serum. *J. Lipid Res.* **2003**, *44*, 705−715.

(19) Staprans, I.; Rapp, J. H.; Pan, X. M.; Feingold, K. R. The Effect of Oxidized Lipids in the Diet on Serum-Lipoprotein Peroxides in Control and Diabetic Rats. *J. Clin. Invest.* **1993**, *92*, 638−643.

(20) Wilson, R.; Lyall, K.; Smyth, L.; Fernie, C. E.; Riemersma, R. A. Dietary hydroxy fatty acids are absorbed in humans: Implications for the measurement of 'oxidative stress' in vivo. *Free Radical Biol. Med.* **2002**, *32*, 162−168.

(21) Penumetcha, M.; Khan-Merchant, N.; Parthasarathy, S. Enhanced solubilization and intestinal absorption of cholesterol by oxidized linoleic acid. *J. Lipid Res.* **2002**, *43*, 895−903.

(22) Frankel, E. N.; Hu, M. L.; Tappel, A. L. Rapid Headspace Gas-Chromatography of Hexanal as a Measure of Lipid-Peroxidation in Biological Samples. *Lipids* **1989**, *24*, 976−981.

(23) Frankel, E. N. Formation of Headspace Volatiles by Thermal-Decomposition of Oxidized Fish Oils Vs Oxidized Vegetable-Oils. *J. Am. Oil Chem. Soc.* **1993**, *70*, 767−772.
(24) Sanches-Silva, A.; Rodriguez-Bernaldo de Quiros, A.; Lopez-Hernandez, J.; Paseiro-Losada, P. Determination of hexanal as indicator of the lipidic oxidation state in potato crisps using gas chromatography and high-performance liquid chromatography. *J. Chromatogr. A* **2004**, *1046*, 75−81.
(25) el-Saadani, M.; Esterbauer, H.; el-Sayed, M.; Goher, M.; Nassar, A. Y.; Jurgens, G. A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. *J. Lipid Res.* **1989**, *30*, 627−630.
(26) Winston, G. W.; Regoli, F.; Dugas, A. J. J.; Fong, J. H.; Blanchard, K. A. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical. Biol. Med.* **1998**, *24*, 480−489.
(27) Privat, C.; Telo, J. P.; Bernardes-Genisson, V.; Vieira, A.; Souchard, J. P.; Nepveu, F. Antioxidant properties of trans-epsilon-viniferin as compared to stilbene derivatives in aqueous and nonaqueous media. *J. Agric. Food Chem.* **2002**, *50*, 1213−1217.
(28) Massaeli, H.; Sobrattee, S.; Pierce, G. N. The importance of lipid solubility in antioxidants and free radical generating systems for determining lipoprotein proxidation. *Free Radical Biol. Med.* **1999**, *26*, 1524−1530.
(29) Morello, J. R.; Vuorela, S.; Romero, M. P.; Motilva, M. J.; Heinonen, M. Antioxidant activity of olive pulp and olive oil phenolic compounds of the arbequina cultivar. *J. Agric. Food Chem.* **2005**, *53*, 2002−2008.
(30) Frankel, E. N. *Lipid Oxidation*; The Oily Press Ltd: Dundee, 1998.
(31) Heinonen, M.; Rein, D.; Satue-Gracia, M. T.; Huang, S. W.; German, J. B.; Frankel, E. N. Effect of protein on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system. *J. Agric. Food Chem.* **1998**, *46*, 917−922.
(32) Reeder, B. J.; Wilson, M. T. Mechanism of reaction of myoglobin with the lipid hydroperoxide hydroperoxyoctadecadienoic acid. *Biochem. J.* **1998**, *330*, 1317−1323.
(33) Reeder, B. J.; Wilson, M. T. The effects of pH on the mechanism of hydrogen peroxide and lipid hydroperoxide consumption by myoglobin: a role for the protonated ferryl species. *Free Radical Biol. Med.* **2001**, *30*, 1311−1318.
(34) Madhujith, T.; Shahidi, F. Antioxidant potential of pea beans (*Phaseolus vulgaris* L.). *J. Food. Sci.* **2005**, *70*, S85−S90.

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