Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations\textsuperscript{1–4}

Zhaoping Li, Susanne M Henning, Yanjun Zhang, Alona Zerlin, Luyi Li, Kun Gao, Ru-Po Lee, Hannah Karp, Gail Thames, Susan Bowerman, and David Heber

\textbf{ABSTRACT}

\textbf{Background:} Emerging science has shown the effect of oxidation products and inflammation on atherogenesis and carcinogenesis. Cooking hamburger meat can promote the formation of malondialdehyde that can be absorbed after ingestion.

\textbf{Objective:} We studied the effect of an antioxidant spice mixture on malondialdehyde formation while cooking hamburger meat and its effects on plasma and urinary malondialdehyde concentrations.

\textbf{Design:} Eleven healthy volunteers consumed 2 kinds of burgers in a randomized order: one burger was seasoned with a spice blend, and one burger was not seasoned with the spice blend. The production of malondialdehyde in burgers and malondialdehyde concentrations in plasma and urine after ingestion were measured by HPLC.

\textbf{Results:} Rosmarinic acid from oregano was monitored to assess the effect of cooking on spice antioxidant content. Forty percent (19 mg) of the added rosmarinic acid remained in the spiced burger (SB) after cooking. There was a 71\% reduction in the malondialdehyde concentration (mean ± SD: 0.52 ± 0.02 µmol/250 g) in the meat of the SBs compared with the malondialdehyde concentration (1.79 ± 0.17 µmol/250 g) in the meat of the control burgers (CBs). The plasma malondialdehyde concentration increased significantly in the CB group as a change from baseline (P = 0.026). There was a significant time-trend difference (P = 0.013) between the 2 groups. Urinary malondialdehyde concentrations (µmol/g creatinine) decreased by 49\% (P = 0.021) in subjects consuming the SBs compared with subjects consuming the CBs.

\textbf{Conclusions:} The overall effect of adding the spice mixture to hamburger meat before cooking was a reduction in malondialdehyde concentrations in the meat, plasma, and urine after ingestion. Therefore, cooking hamburgers with a polyphenol-rich spice mixture can significantly decrease the concentration of malondialdehyde, which suggests potential health benefits for atherogenesis and carcinogenesis. This trial was registered at clinicaltrials.gov as NCT01027052. \textit{Am J Clin Nutr} 2010;91:1180–4.

\textbf{INTRODUCTION}

Over the past 30 y, there has been accumulating evidence that lipid oxidation can play an important role in the processes of atherogenesis and carcinogenesis. Specific proinflammatory oxidized phospholipids that result from the oxidation of LDL phospholipids containing arachidonic acid are recognized by the innate immune system in animals and humans and lead to inflammation, which can promote atherogenesis and carcinogenesis.

Fogelman et al (1) reported that malondialdehyde, an obligate product of the oxidation of arachidonic acid by lipoxygenase pathways, could cause Schiff’s base formation with the \(\epsilon\) amino groups of apolipoprotein B lysine residues in LDL. Altered lipoproteins bind to macrophage scavenger receptors, resulting in cholesteryl ester accumulation and the formation of foam cells. Oxidatively modified LDL is present in the artery walls of animals and humans with atherosclerosis and leads to destabilization of atherosclerotic plaques (2–4).

Malondialdehyde can also react with deoxyadenosine and deoxyguanosine in DNA and form DNA adducts that are mutagenic. Thus, the formation of malondialdehyde has implications for atherogenesis and carcinogenesis (5). Inhibition of the formation of malondialdehyde by antioxidants during the cooking of hamburger meat may result in reduced concentrations of malondialdehyde in plasma and urine as the result of inhibition of malondialdehyde formation ex vivo or the inhibition of its formation or absorption from the gastrointestinal tract in vivo (6, 9). Such a reduction would suggest that the processes of lipid peroxidation and DNA adduct formation could be reduced (7–9).

Recently, Gorelik et al (9) observed that the addition of red-wine polyphenols to turkey meat and consumption of red wine with turkey meat could inhibit the absorption of malondialdehyde from cooked turkey meat in humans. The current study was conducted to determine the effects of adding a polyphenol-rich spice mixture containing rosmarinic acid to hamburger meat before cooking to establish the effects on the absorption of cytotoxic lipids as determined by measuring malondialdehyde concentrations in plasma and urine.

\textsuperscript{1} From the University of California, Los Angeles Center for Human Nutrition, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA.

\textsuperscript{2} Other than the preparation of the research-grade spice mixture, no one from McCormick and Company Inc had any role in the conduct of the study, analysis of the data, or preparation of the manuscript.

\textsuperscript{3} Supported by the University of California, Los Angeles General Clinical Research Center (National Institutes of Health grant RR-00865) and the Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles.

\textsuperscript{4} Address correspondence to D Heber, University of California, Los Angeles Center for Human Nutrition, 900 Veteran Avenue, Room 12-217, Warren Hall, Box 951742, Los Angeles, CA 90095. E-mail: dheber@mednet.ucla.edu.

Received August 12, 2009. Accepted for publication February 5, 2010. First published online March 24, 2010; doi: 10.3945/ajcn.2009.28526.
SUBJECTS AND METHODS

The study used a prospectively randomized crossover design and initially included 11 healthy volunteers. One subject dropped out after screening but before the intervention. The study protocol was approved by the University of California, Los Angeles (UCLA), Institutional Review Board. All subjects gave written informed consent before the study procedures were conducted. Subjects were excluded if they had metabolic disorders, were taking dietary supplements, smoked >1 cigarette/d, exercised heavily (aerobic exercise >4 times for 30 min/wk), or drank >2 alcoholic beverages/d. Each subject was seen at the UCLA Center for Human Nutrition Clinical Research Unit on 2 separate occasions separated by ≥1 wk. Subjects consumed, in a random order, 2 different test meals consisting of either 1) a cooked ground-beef burger (control) or 2) a ground beef burger seasoned with a spice mixture during cooking. The subjects were asked to avoid eating meat, poultry, or fish products for the 3 d immediately before each of the 2 testing visits.

Hamburger patties were prepared in the kitchen of the UCLA General Clinical Research Center. The beef was weighed, minced in a large Kitchen Aid mixer bowl (Hobart model M-802; Hobart Corp, Troy, OH) for 2 min on the lowest setting, and mixed with either 1 g salt alone or 1 g salt plus 11.25 g spice mix/250 g meat (Table 1). A research-grade spice blend was purchased from the McCormick Science Institute, Hunt Valley, MD. The constituents of the spice mix are listed in Table 2. After blending with the paddle attachment for 1 min, a 5.75-in ring mold was used to divide the meat into flattened 250-g patties. The burger patties were cooked to an internal temperature of 77°C, frozen, and packaged in the UCLA General Clinical Research Center kitchen. They were delivered frozen to the UCLA Center for Human Nutrition. The patties were then reheated in their packaging with a double boiler at the UCLA Center for Human Nutrition to ~60°C as needed for intervention visits.

For each intervention visit, the subjects were asked to report to the UCLA Center for Human Nutrition in a fasting state. An indwelling catheter was inserted into a forearm vein, and a baseline blood sample was collected into EDTA-treated tubes.



| Spice                     | Percentage | Weight g/burger |
|---------------------------|------------|-----------------|
| Cloves, ground            | 4.34       | 0.5             |
| Cinnamon, ground          | 4.34       | 0.5             |
| Oregano, Mediterranean, ground | 26.17   | 3.0             |
| Rosemary, ground          | 4.34       | 0.5             |
| Ginger, ground            | 10.86      | 1.2             |
| Black pepper, ground      | 6.51       | 0.7             |
| Paprika, ground           | 30.44      | 3.4             |
| Garlic powder             | 12.99      | 1.5             |
| Total                     | 100.0      | 11.3            |

The study used a prospectively randomized crossover design and initially included 11 healthy volunteers. One subject dropped out after screening but before the intervention. The study protocol was approved by the University of California, Los Angeles (UCLA), Institutional Review Board. All subjects gave written informed consent before the study procedures were conducted. Subjects were excluded if they had metabolic disorders, were taking dietary supplements, smoked >1 cigarette/d, exercised heavily (aerobic exercise >4 times for 30 min/wk), or drank >2 alcoholic beverages/d. Each subject was seen at the UCLA Center for Human Nutrition Clinical Research Unit on 2 separate occasions separated by ≥1 wk. Subjects consumed, in a random order, 2 different test meals consisting of either 1) a cooked ground-beef burger (control) or 2) a ground beef burger seasoned with a spice mixture during cooking. The subjects were asked to avoid eating meat, poultry, or fish products for the 3 d immediately before each of the 2 testing visits.

Hamburger patties were prepared in the kitchen of the UCLA General Clinical Research Center. The beef was weighed, minced in a large Kitchen Aid mixer bowl (Hobart model M-802; Hobart Corp, Troy, OH) for 2 min on the lowest setting, and mixed with either 1 g salt alone or 1 g salt plus 11.25 g spice mix/250 g meat (Table 1). A research-grade spice blend was purchased from the McCormick Science Institute, Hunt Valley, MD. The constituents of the spice mix are listed in Table 2. After blending with the paddle attachment for 1 min, a 5.75-in ring mold was used to divide the meat into flattened 250-g patties. The burger patties were cooked to an internal temperature of 77°C, frozen, and packaged in the UCLA General Clinical Research Center kitchen. They were delivered frozen to the UCLA Center for Human Nutrition. The patties were then reheated in their packaging with a double boiler at the UCLA Center for Human Nutrition to ~60°C as needed for intervention visits.

For each intervention visit, the subjects were asked to report to the UCLA Center for Human Nutrition in a fasting state. An indwelling catheter was inserted into a forearm vein, and a baseline blood sample was collected into EDTA-treated tubes. The subjects were provided with either the spiced burger (SB) or a cooked ground-beef burger (control) or 2) a ground beef burger seasoned with a spice mixture during cooking. The subjects were asked to avoid eating meat, poultry, or fish products for the 3 d immediately before each of the 2 testing visits.

Hamburger patties were prepared in the kitchen of the UCLA General Clinical Research Center. The beef was weighed, minced in a large Kitchen Aid mixer bowl (Hobart model M-802; Hobart Corp, Troy, OH) for 2 min on the lowest setting, and mixed with either 1 g salt alone or 1 g salt plus 11.25 g spice mix/250 g meat (Table 1). A research-grade spice blend was purchased from the McCormick Science Institute, Hunt Valley, MD. The constituents of the spice mix are listed in Table 2. After blending with the paddle attachment for 1 min, a 5.75-in ring mold was used to divide the meat into flattened 250-g patties. The burger patties were cooked to an internal temperature of 77°C, frozen, and packaged in the UCLA General Clinical Research Center kitchen. They were delivered frozen to the UCLA Center for Human Nutrition. The patties were then reheated in their packaging with a double boiler at the UCLA Center for Human Nutrition to ~60°C as needed for intervention visits.

For each intervention visit, the subjects were asked to report to the UCLA Center for Human Nutrition in a fasting state. An indwelling catheter was inserted into a forearm vein, and a baseline blood sample was collected into EDTA-treated tubes. The subjects were provided with either the spiced burger (SB) or the control burger (CB) to be eaten within 30 min along with a glass of water (200 mL). Blood samples were drawn every hour for 6 h after the meal was eaten, and all urine was collected for measurement of malondialdehyde and creatinine. Plasma was separated from whole blood by centrifugation (910 × g for 15 min), frozen, and kept at −80°C for <1 wk until determination of malondialdehyde concentrations by HPLC.

Rosmarinic acid, a potent antioxidant polyphenol in the spice mixture, was quantified in the spice mixture and in the SBs before and after cooking as follows. A rosmarinic acid standard (1 mg) was dissolved in 1 mL methanol as a stock solution. A total of 500 μL of the standard stock solution was further diluted to afford 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL concentrations. A standard calibration curve was constructed for a rosmarinic acid standard. The burger sample’s rosmarinic acid content was measured from the peak area and the linear regression equations obtained from the calibration curve. Fifty milligrams of the blended spice sample was extracted in 10 mL methanol by sonication for 20 min with an ice pack in the water bath. The solutions were diluted 10-fold in water and centrifuged at 11,800 × g for 5 min. The supernatant fluid was loaded into the HPLC for analysis after filtering through a 0.22-μm membrane.

The average cooked burger weighed ~170 g. A quarter of the burger (40 g) was cut from a whole burger and blended with a food blender. Ten grams of the blended burger sample was extracted by sonication with 100 mL MeOH for 30 min. The extraction mixture was centrifuged at 2600 × g for 15 min. The supernatant fluid was separated from the residue by transferring the liquid into a flask. The same extraction procedure was repeated 3 times; the supernatant fluids from 3 extractions were combined and the volume condensed under reduced pressure at 50°C. The remaining residue solution was transferred into a 100-mL volumetric flask. The flask used to condense the volume was rinsed 3 times with 5 mL methanol each, and the rinse was combined in the volumetric flask. Methanol was added into the volumetric flask to the volume. The solution was centrifuged at 11,300 × g, and the supernatant fluid was filtered through a 0.22-μm membrane filter for HPLC analysis. The HPLC system consisted of a Waters Alliance 2695 Module with a 996 PDA (photodiode array) detector, which was controlled by Waters Empower 2 Software (Waters, Milford, MA). The mobile phase, solvent A (acetonitrile) and solvent B (0.4% aqueous phosphoric acid), was used under binary linear-gradient conditions as follows: 1–30% of solvent A in solvent B for 0–60 min and 31–40% of solvent A in solvent B for 60–70 min, with a flow rate of 0.75 mL/min. All samples were filtered (0.22 μm), loaded (25 μL injection volume), and analyzed on an Agilent Zorbax SB C18, 4.6 × 250-mm column (Agilent Technology, Wilmington, DE) with a guard column (C18 5 μm, 3.9 × 20 mm). The monitored wavelength was 330 nm for detection and quantification of the rosmarinic acid.

### Table 1
Composition of the study burgers

| Component                        | Spiced burger | Control burger |
|----------------------------------|---------------|----------------|
| Ground beef, 10% fat             | 236.3 g       | 247.5 g        |
| Salt                             | 2.5 g         | 2.5 g          |
| Seasoning blend                  | 11.25 g       | 0 g            |
| Raw burger patty weight          | 250 g         | 250 g          |

1 The University of California, Los Angeles General Clinical Research Center, prepared the study burgers as described. Twenty burgers were prepared with salt only, and 20 burgers were prepared with salt and spice. Measurements were taken with an accuracy to 0.1 g.
Thiobarbituric acid (TBA) reactive substances were reported as the malondialdehyde concentration, which was measured in plasma, urine, and the meat homogenate by alkaline hydrolysis, acid deproteinization, derivatization with TBA, and n-butanol extraction according to the method of Grotto et al (10). In brief, 75 μL plasma was mixed with 25 μL 3 N NaOH as well as 25 μL water and incubated in a 60°C water bath for 45 min. The hydrolyzed sample was acidified with 125 μL of 6% H3PO4 and 125 μL 0.8% TBA. After heating at 90°C for 45 min, 50 μL 10% sodium dodecyl sulfate was added to the cool-down sample, and malondialdehyde-TBA2 adduct was extracted with 300 μL n-butanol. After centrifugation, 50 μL supernatant fluid was directly injected into the HPLC system for analysis. Chromatographic determinations were performed on an Agilent 1100 series HPLC (Agilent Technology) equipped with a Varian 9070 fluorescence detector (Varian Inc, Lake Forest, CA) at Ex (excitation) 530 nm and Em (emission) 550 nm. An Alltima C18 Guard column (Alltech, Deerfield, IL) and YMC-Pack Octademy Silane-AQ C18 reversed-phase column (15 cm, 4.6-mm inside diameter; 5-μm particles; Waters, Milford, MA) was used for separation at an ambient temperature. A linear gradient of methanol in water (from 50% to 100% in 10 min) at a flow rate of 0.8 mL/min was used for the elution. The reagent 1,1,3,3-tetramethoxypropane was used to prepare a standard curve.

Data were expressed as means ± SDs. A log transformation was applied to the concentrations of plasma malondialdehyde to satisfy the normality assumption of the model. A linear mixed-effects model to evaluate the effect of meal type on the concentration of malondialdehyde was used. The fixed effects in the model included meal type, time, and the meal type/time interaction. A random intercept was used to accommodate the correlation from the same subject. After imputation, we calculated the ratio of malondialdehyde at each time point to its baseline value for each subject.

The primary statistical analysis tool was the generalized estimating equation (GEE) method to analyze the repeated measurements of the plasma malondialdehyde concentration for its robust statistical inference. The missing values were imputed with SAS proc multiple imputation procedures (SAS Institute, Chicago, IL) after log transformation to satisfy the normal distribution assumption of imputation. A GEE was used to analyze the repeated ratios of each subject with an unstructured working-covariance matrix. Differences were considered significant at \( P < 0.05 \). The SAS 9.1.3 software package (SAS Institute) was used for analyses.

RESULTS

Rosmarinic acid is a typical marker for 2 of the ingredients of the spice mix: rosemary and oregano. With the use of HPLC, we determined that the 11.25-g spice mix, which was added to each hamburger, contained 48.4 mg rosmarinic acid (Tables 1 and 2). During the cooking process, the content of rosmarinic acid decreased to 18.5 ± 0.4 mg whereas the burger weight decreased from 250 g (raw) to 170 g (cooked). The malondialdehyde content in the cooked burger was significantly decreased by 71% with the addition of the spice mix (1.79 ± 0.17 μmol/250 g meat in the CB compared with 0.52 ± 0.02 μmol/250 g meat in the SB; \( P < 0.05 \)) (Figure 1).

Eleven healthy subjects were recruited and enrolled in this study (Table 3). One subject dropped out of the study because of a time conflict. No side effect was observed. The mean fasting plasma malondialdehyde concentration was 6.26 ± 3.49 μmol/L. The postprandial concentration of plasma malondialdehyde increased significantly in all subjects after consumption of the CB (\( P = 0.043 \)), whereas the malondialdehyde concentration showed a trend to decrease after consumption of the SBs. After consumption of the CB, plasma malondialdehyde concentration, which was calculated as the change from baseline, increased significantly (\( P = 0.026 \)). With the use of the GEE primary analysis tool, we determined a significant time-trend difference (\( P = 0.013 \)) between the 2 groups (Figure 2).

Although the contributions of digestion and metabolism to malondialdehyde formation were not determined, there was clearly a reduction in plasma malondialdehyde concentrations in the SB group. More striking differences were seen when urinary malondialdehyde excretion was considered. The malondialdehyde concentration in μmol/g creatinine was reduced by 49% (\( P = 0.021 \)) in the subjects who consumed the SBs compared with subjects who consumed the CBs (Figure 3).

An evaluation of the preference of the participants to consume the CB compared with the SB was performed by using the hedonic scale of 1–9 from like extremely (9) to dislike extremely (1). There was a trend for the participants to prefer the CB, but the difference between the 2 hamburgers was not significant (hedonic score for the SB: 4.8 ± 2.6; hedonic score for the CB: 6.3 ± 1.3; \( P = 0.06 \)).

DISCUSSION

Oxidative stress and inflammation are integral aspects of atherosclerosis and carcinogenesis as well as of other age-related chronic diseases (1, 2, 11). The ingestion of high-fat foods that contain lipid-peroxidation products can lead to increases in

![FIGURE 1. Mean (±SD) malondialdehyde production in the burgers after cooking (n = 2). *P = 0.009 between the 2 groups.](https://academic.oup.com/ajcn/article-abstract/91/5/1180/4597198/FIGURE-1-Mean-±SD-malondialdehyde-production-in-the-burgers-after-cooking-n-2P-0.009-between-the-2-groups)

**TABLE 3**

| Characteristic | Values |
|---------------|--------|
| Total subjects (n) | 11 |
| Men [n (%)] | 6 (55) |
| Women [n (%)] | 5 (45) |
| Age (y) | 31.3 ± 2.5* |
| Race [n (%)] | |
| Asian | 1 (9.1) |
| Black | 4 (36.4) |
| White | 4 (36.4) |
| Hispanic | 1 (9.1) |
| Other | 1 (9.1) |
| BMI (kg/m²) | 25.6 ± 1.4 |

*Mean ± SE (all such values).
There was a significant time-trend difference between the 2 groups (baseline increased significantly in the control burger group. The baseline plasma MDA concentration was $5.8 \pm 3.1 \mu mol/L$ for the control burger and $8.1 \pm 4.2 \mu mol/L$ for the spiced burger. *Plasma MDA as percentage change from baseline increased significantly in the control burger group ($P = 0.026$). **There was a significant time-trend difference between the 2 groups ($P = 0.013$).

plasma concentrations of malondialdehyde as well as other cytotoxic and genotoxic compounds (12–16). Most of the lipid-peroxidation products ingested from popular foods in the United States are derived from meat products and high-fat processed foods (17). The use of antioxidants from dietary sources, including herbs and spices, to prevent lipid oxidation has been proposed as an adjunct to other preventive measures such as achieving and maintaining a healthy body weight and controlling blood cholesterol concentrations with drugs (12). Among commonly eaten foods, spices have the highest known concentrations of antioxidant and antiinflammatory polyphenols that have the potential to inhibit the oxidation of LDL (18–22). The intake of polyphenols in a well-balanced diet that includes the recommended daily servings of fruit and vegetables and spice could potentially reduce the formation of malondialdehyde and urinary malondialdehyde after ingestion of the SB compared to after ingestion of the CB. We observed a decrease in the excretion of plasma malondialdehyde and urinary malondialdehyde after ingestion of the SB compared to after ingestion of the CB.

After ingestion of lipid-peroxidation products, increased amounts of malondialdehyde are excreted in the urine (5, 31, 32). Lipids can undergo oxidation in the stomach (33), which leads to the formation of malondialdehyde. Because the gastrointestinal tract is exposed to lipid-oxidation products during digestion and absorption (12, 34), Halliwell (35) proposed that dietary antioxidants may protect against the deleterious effects of oxidation products in the gastrointestinal tract. Studies (36–38) showed interactions of dietary polyphenols and oxidation products found in the gastrointestinal tract. Therefore, the benefits of consuming plant polyphenols as an integral part of a meal in which meat products are eaten may derive from inhibition of the formation and absorption of malondialdehyde and other lipid-peroxidation products (6, 7, 33). Although the contribution of the antioxidant spice mix to malondialdehyde formation during digestion could not be determined with our study design, studies by other investigators (6, 9) provided preliminary evidence of the protective effect of antioxidant supplements during the digestive process.

Foods such as cooked hamburger meat containing oxidized products affect endogenous lipid metabolism and can lead to excess lipid-peroxidation product exposure that leads to the promotion of the multistep processes of atherogenesis and carcinogenesis. Although there is a great deal of evidence suggesting that healthy diets that are low in fat and refined sugars and rich in colorful fruit and vegetables can reduce the risk of heart disease and common forms of cancer, there is less evidence on the effects of adding spice to commonly eaten foods known to contain lipid-peroxidation products such as red meats cooked at high temperatures. This study showed that spices that are rich in antioxidants may be useful when cooking meat products to reduce the formation of lipid-peroxidation products. The results also suggest that the lower concentrations of malondialdehyde observed in plasma and urine after ingestion of meat products seasoned heavily with antioxidant-rich spices may lead to reduced in vivo formation and action of lipid-peroxidation products relevant to the oxidant stress-related risk of heart disease and common forms of cancer.

The authors’ responsibilities were as follows—ZL: supervised the clinical intervention trial and prepared the manuscript; SMH: conducted laboratory analyses and contributed substantially to the preparation of the manuscript; YZ: supervised the chemical analysis of the spice mix; AZ: provided dietary advice and study coordination; LL and KG: performed malondialdehyde analyses; R-PL: performed the spice analysis; HK: assisted in the study coordination; GT: completed the approval process of the institutional review board; SB: optimized the burger preparation and composition of the spice mix; and...
REFERENCES

1. Vogtman AM, Shechter I, Seager J, Hokom M, Child JS, Edwards PA. Malondialdehyde alteration of low density lipoproteins leads to cholesterol ester accumulation in human monocyte-macrophages. Proc Natl Acad Sci USA 1980;77:2214–8.

2. Haberland ME, Fong D, Cheng L. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. Science 1988;241:215–8.

3. Palinski W, Rosenfeld ME, Yla-Herttuala S, et al. Low density lipoprotein undergoes oxidative modification in vivo. Proc Natl Acad Sci USA 1989;86:1376–6.

4. Yla-Herttuala S, Palinski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J Clin Invest 1989;84:1086–95.

5. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res 1999;424:83–95.

6. Gorelik S, Ligumsky M, Kohen R, Kanner J. The stomach as a "bioreactor": when red meat meets red wine. J Agric Food Chem 2008;56:5002–7.

7. Grotto D, Santa Maria LD, Boeira S, et al. Rapid quantification of microns of diabetic patients. Diabetes Care 1999;22:300–6.

8. Yla-Herttuala S, Glatt H, Muckel E, Platt KL. Protection by wine polyphenols in humans: prevention of absorption of cytotoxic lipid oxidation process in stomach medium. Arch Biochem Biophys 2007;458:236–43.

9. Gorelik S, Ligumsky M, Kohen R, Kanner J. A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products. FASEB J 2008;22:41–6.

10. Grotto D, Santa Maria LD, Boeira S, et al. Rapid quantification of malondialdehyde in plasma by high performance liquid chromatography-visible detection. J Pharm Biomed Anal 2007;43:619–24.

11. Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Lett 1995;358:1–3.

12. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. J Nutr 2005;135:969–72.

13. Domar HJ, Bachmayer O, Kosar M, Hilutnen R. Antioxidant properties of aqueous extracts from selected lamiaceae species grown in Turkey. J Agric Food Chem 2004;52:762–70.

14. Stapanian I, Hardman DA, Pan XM, Feingold KR. Effect of oxidized lipids in the diet on oxidized lipid levels in postprandial serum chyomicrons of diabetic patients. Diabetes Care 1999;22:300–6.

15. Williams MJ, Sutherland WH, McCormick MP, de Jong SA, Walker RJ, Wilkins GT. Impaired endothelial function following a meal rich in used cooking fat. J Am Coll Cardiol 1999;33:1050–5.

16. Ursini F, Zamburlini A, Cazzolato G, Maiorino M, Bon GB, Sevanian A. Postprandial plasma lipid hydroperoxides: a possible link between diet and atherosclerosis. Free Radic Biol Med 1998;25:250–2.

17. Goldberg T, Cai W, Peppe M, et al. Advanced glycoxidation end products in commonly consumed foods. J Am Diet Assoc 2004;104:1287–91.

18. Halvorsen BL, Holte K, Myhrstad MC, et al. A systematic screening of total antioxidants in dietary plants. J Nutr 2002;132:461–71.

19. Kris-Etherton PM, Hecker KD, Bonanome A, et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002;113(suppl 9B):71S–88S.

20. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr 2003;133:1286–90.

21. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 2005;45:287–306.

22. Konishi Y, Kobayashi S. Transepithelial transport of rosmarinic acid in intestinal Caco-2 cell monolayers. Biosci Biotechnol Biochem 2005;69:585–91.

23. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med 2006;41:1727–46.

24. Kuhnau J. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev Nutr Diet 1976;24:117–91.

25. Aviram M, Dornfeld L, Kaplan M, et al. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drugs Exp Clin Res 2002;28:49–62.

26. Bors W, Saran M. Radical scavenging by flavonoid antioxidants. Free Radic Res Commun 1987;2:289–94.

27. Cuevas AM, Guasch V, Castillo O, et al. A high-fat diet induces and red wine counteracts endothelial dysfunction in human volunteers. Lipids 2000;35:143–8.

28. Woo HM, Kang JH, Kawada T, Yoo H, Sung MK, Yu R. Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. Life Sci 2007;80:926–31.

29. Edenharder R, Sager JW, Glatt H, Muckel E, Platt KL. Protection by beverages, fruits, vegetables, herbs, and flavonoids against genotoxicity of 2-acetylaminoflourene and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in metabolically competent V79 cells. Mutat Res 2002;521:57–72.

30. Martinez-Tome M, Jimenez AM, Ruggieri S, Frega N, Strabbioli R, Murcia MA. Antioxidant properties of Mediterranean spice compared with common food additives. J Food Prot 2001;64:1412–9.

31. Brown ED, Morris VC, Rhodes DG, Sinha R, Levander OA. Urinary malondialdehyde-equivalents during ingestion of meat cooked at high or low temperatures. Lipids 1995;30:1053–6.

32. Stapanian I, Pan XM, Rapp JH, Feingold KR. Oxidized cholesterol in the diet is a source of oxidized lipoproteins in human serum. J Lipid Res 2003;44:705–15.

33. Kanner J, Lapidot T. The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. Free Radic Biol Med 2001;31:1388–95.

34. Kubow S. Routes of formation and toxic consequences of lipid oxidation products in foods. Free Radic Biol Med 1992;12:63–81.

35. Halliwell B, Zhao K, Whiteman M. The gastrointestinal tract: a major site of antioxidant action? Free Radic Res 2000;33:819–30.

36. Tottani VM, Peterson DG. Epicatechin carboxyl-trapping reactions in aqueous maillard systems: identification and structural elucidation. J Agric Food Chem 2006;54:7311–8.

37. Lo CY, Li S, Tan D, Pan MH, Sang S, Ho CT. Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. Mol Nutr Food Res 2006;50:1118–28.

38. Piche LA, Cole PD, Hadley M, van den BR, Draper HH. Identification of N-episal din(2-propanenyl)lysine as the main form of malondialdehyde in food digesta. Carcinogenesis 1988;9:473–7.