Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations[1–4]

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ABSTRACT

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.

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.

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.

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.

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 clinical trials.gov as NCT01027052. *Am J Clin Nutr* 2010;91:1180–4.

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 ε 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.

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[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.

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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. 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.

TABLE 1
Composition of the study burgers[1]

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

[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.

TABLE 2
Composition of the spice mixture

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

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 sonicating 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 sonicating 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 C_{18} 4.6 × 250-mm column (Agilent Technology, Wilmington, DE) with a guard column (C_{18} 5μm, 3.9 × 20 mm). The monitored wavelength was 330 nm for detection and quantification of the rosmarinic acid.

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 hydrolized sample was acidified with 125 μL of 6% H_3PO_4 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-$(TBA)_2$ 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. An Alltima C18 Guard column (Alltech, Deerfield, IL) and YMC-Pack Octadedyl 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**).

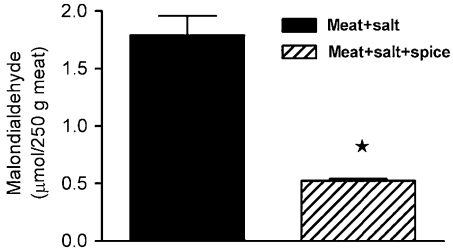

FIGURE 1. Mean (±SD) malondialdehyde production in the burgers after cooking ($n = 2$). *$P = 0.009$ between the 2 groups.

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

TABLE 3
Subject characteristics

Characteristic	Values
Total subjects (*n*)	11
Men [*n* (%)]	6 (55)
Women [*n* (%)]	5 (45)
Age (y)	31.3 ± 2.5[1]
Race [*n* (%)]	
Asian	1 (9.1)
Black	4 (36.4)
White	4 (36.4)
Hispanic	1 (9.1)
Other	1 (9.1)
BMI (kg/m^2)	25.6 ± 1.4

[1] Mean ± SE (all such values).

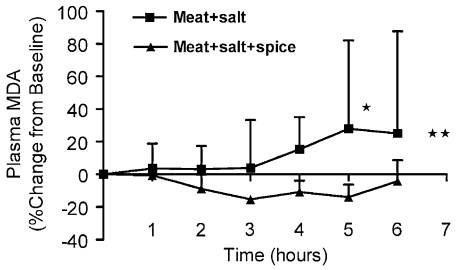

FIGURE 2. Mean (±SD) plasma malondialdehyde (MDA) as percentage change (%Change) from baseline (n = 10). The baseline plasma MDA concentration was 5.8 ± 3.1 μmol/L for the control burger and 8.1 ± 4.2 μmol/L for the spiced burger. *Plasma MDA as percentage change from baseline increased significantly 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 easily exceed 1000 mg/d (23, 24). Some reported biological effects of polyphenols include antioxidant activity (25, 26), improvement of endothelial function (27), inhibition of inflammation (28), and stimulation of DNA repair mechanisms (29).

The composition of the spice mix used in this study is similar to some of the spice blend in East Indian, and they were selected for their taste, flavor, and antioxidant capacity with the aim to have the total antioxidant capacity from whole spice mix to reach 10,000 Trolox equivalents/250 g burger. The 2 key spices in our mixture, rosemary and oregano, have the strongest ability to inhibit lipid peroxidation (30). Although the amount of the spice mixture added to the hamburger meat was well above the amounts usually added to American-style burgers, participants readily accepted the taste of the SBs. This study showed that adding spice to hamburger meat before cooking significantly reduced the formation of lipid-peroxidation products during cooking.

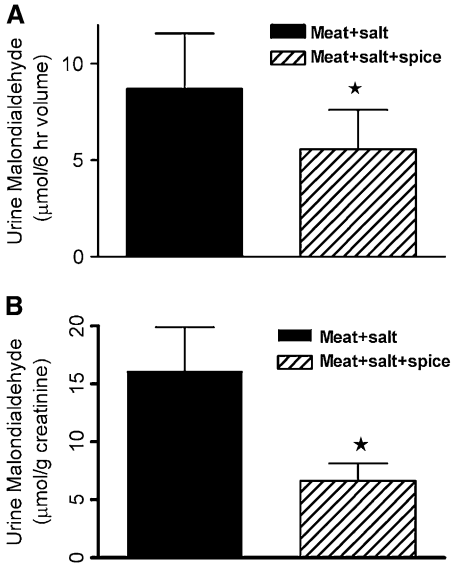

FIGURE 3. Mean (±SD) accumulation of malondialdehyde in the urine expressed as μmol/6 h urine (A) and as μmol/g creatinine (B) (n = 10). *$P <$ 0.05 between the 2 groups.

After ingestion of lipid-peroxidation products, increased amounts of malondialdehyde are excreted in the urine (5, 31, 32). 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.

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-oxidation 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

DH: created the intellectual study design and conducted the overall data interpretation. DH is a member of the Nutrition Advisory Board of the McCormick Science Institute and has received honoraria from the McCormick Science Institute. The McCormick Science Institute is a nonprofit unit of McCormick and Company Inc, which is dedicated to promoting research on spices. ZL, SMH, YZ, AZ, LL, KG, R-PL, HK, GT, and SC had no conflicts of interest.

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