

## Effects of Capsaicin, Dihydrocapsaicin, and Curcumin on Copper-Induced Oxidation of Human Serum Lipids

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The oxidation of low-density lipoprotein (LDL) is believed to be the initiating factor for the development and progression of atherosclerosis. The active ingredients of spices such as chili and turmeric (capsaicin and curcumin, respectively) have been shown to reduce the susceptibility of LDL to oxidation. One of the techniques used to study the oxidation of LDL is to isolate LDL and subject it to metal-induced (copper or iron) oxidation. However, whole serum may represent a closer situation to in vivo conditions than using isolated LDL. We investigated the effects of different concentrations (0.1–3  $\mu\text{M}$ ) of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of serum lipoproteins. The lag time (before initiation of oxidation) and rate of oxidation (slope of propagation phase) were calculated. The lag time increased, and the rate of oxidation decreased with increasing concentrations of the tested antioxidants ( $p < 0.05$ ). A 50% increase in lag time (from control) was observed at concentrations between 0.5 and 0.7  $\mu\text{M}$  for capsaicin, dihydrocapsaicin, and curcumin. This study shows that oxidation of serum lipids is reduced by capsaicinoids and curcumin in a concentration-dependent manner.

**KEYWORDS:** LDL oxidation; chilli; turmeric; spice; antioxidant

### INTRODUCTION

Oxidation of low-density lipoprotein (LDL) is believed to contribute to the development and progression of atherosclerosis. Highly cytotoxic oxidized LDL induces changes in endothelial cells and enhances proliferation of monocytes and smooth muscle cells (1, 2). Chili extracts, capsaicin (the active ingredient of chili), and other capsaicinoids (such as capsiate and dihydrocapsiate), when incubated with isolated LDL cholesterol and/or oils and fats, increase their resistance to oxidation by delaying the initiation and/or slowing the rate of oxidation (3–5). Similarly, curcumin, the active ingredient of turmeric, has been shown to increase the resistance of LDL to oxidation (5). Capsaicin, dihydrocapsaicin, and curcumin are fat-soluble compounds (6). In rats, the absorption of the capsaicinoids has been found to be about 60–80% (7), while that of curcumin has been found to be about 13–60% (8, 9).

A common procedure used for measuring the resistance of LDL to in vitro oxidation is to determine the lag time for conjugated diene formation. Using isolated LDL as an indicator of in vivo resistance to oxidation has limitations because of the absence of the serum water-soluble antioxidants and pro-oxidants. These may be important in resisting or augmenting the oxidation process. Similarly, high-density lipoprotein (HDL) with antioxidative, anti-inflammatory, and antiaggregatory

properties (10) is also removed when testing isolated LDL. Hence, whole serum used for oxidation tests may provide a better representation of the in vivo situation than isolated LDL cholesterol.

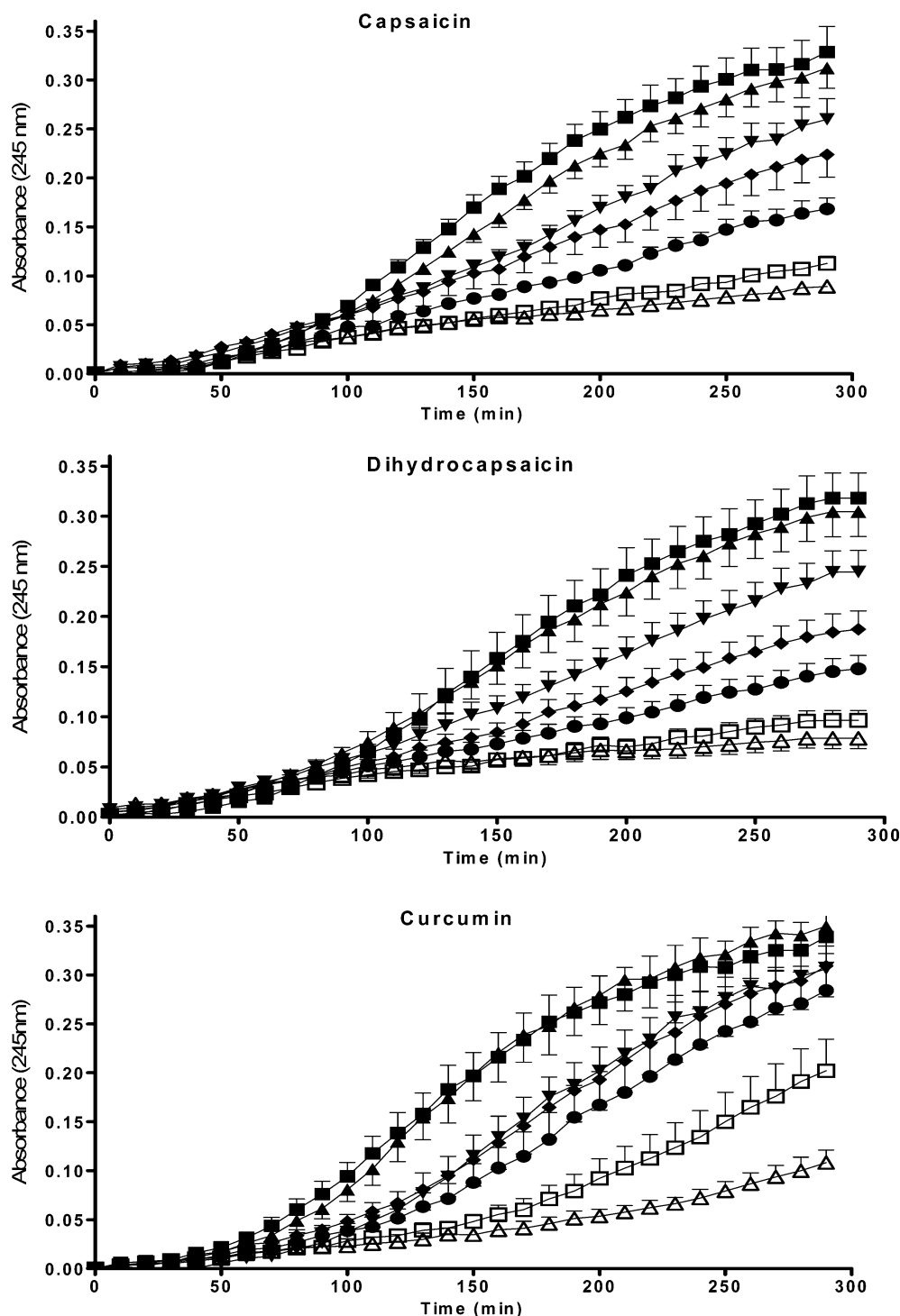
In the present study, we investigated the effect of different concentrations of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of lipids in serum.

### MATERIALS AND METHODS

Fasting venous blood samples from six healthy individuals (three men and three women; mean age,  $34 \pm 10$  years), collected in tubes without anticoagulant, were allowed to clot in the dark at room temperature and were then centrifuged at 2500 rpm (1335g) at 4 °C for 20 min. The separated serum was frozen at  $-80$  °C for later analysis of serum lipoprotein oxidation.

Copper-induced oxidation of serum was undertaken using the method described by Schnitzer et al. (11). Briefly, serum was thawed and diluted 50-fold in phosphate-buffered saline (pH 7.4), incubated with increasing concentrations (0.1, 0.5, 0.7, 1, 2, and 3  $\mu\text{M}$ ) of capsaicin (purity  $\geq 98.5\%$ ; Tocris, United States), dihydrocapsaicin (purity  $\geq 90\%$ ; Fluka, Switzerland), and curcumin (purity  $\geq 95\%$ ; Fluka), and subjected to copper (100  $\mu\text{M}$ )-induced oxidation. Oxidation kinetics were determined for each serum sample in duplicate by measuring the absorbance at 245 nm at 37 °C using a multiposition spectroscope (Cintra 10E UV-vis, GBC scientific equipments, Victoria, Australia) every 10 min for 300 min. Each run of 300 min consisted of two control samples and four samples (two duplicates for two different concentrations) of

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**Figure 1.** Copper-induced oxidation curves for serum with different concentrations (■, 0  $\mu\text{M}$ ; ▲, 0.1  $\mu\text{M}$ ; ▼, 0.5  $\mu\text{M}$ ; ◆, 0.7  $\mu\text{M}$ ; ●, 1  $\mu\text{M}$ ; □, 2  $\mu\text{M}$ ; and △, 3  $\mu\text{M}$ ) of capsaicin, dihydrocapsaicin, and curcumin. Values are shown as means  $\pm$  SEM for six duplicate determinations with each concentration of capsaicin, dihydrocapsaicin, and curcumin.

antioxidants. Absorbance data were plotted against time. Lag time, an indicator of the resistance of the serum lipoproteins to oxidation, was calculated as the intercept between the baseline (time zero) and the tangent of the absorbance curve during the propagation phase. The rate of oxidation was calculated as the slope of the propagation phase. The maximum change in absorbance was calculated as the absorbance difference between time zero and time 300 min.

Freeze-thawing of serum may affect the natural defense of the serum. However, our studies have shown that freezing for up to 12 weeks at  $-80^\circ\text{C}$  has no significant effect in the kinetic profile of oxidation as compared with fresh serum (data not shown). Similar results were reported by Schnitzer et al. (12) for serum stored at  $-15^\circ\text{C}$ .

To avoid any investigator bias, the lag phase (before initiation of oxidation) and slope of the propagation phase (rate of oxidation) were calculated by an observer who was blinded to the individual compounds and concentrations. Repeated measurements of analysis of variance (ANOVA) using general linear modeling (GLM) (STATA version 8.2, StataCorp LP, United States) were used to test for any differences between the controls and the individual concentrations of capsaicin, dihydrocapsaicin, and curcumin. Because of the small sample size, the results were also confirmed with OLOGIT analysis, the statistical test used for nonparametric data. The data are presented as means  $\pm$  standard deviations unless otherwise reported.

## RESULTS

Oxidation curves for serum incubated with increasing concentrations of capsaicin, dihydrocapsaicin, and curcumin are shown in **Figure 1**. A decrease in overall absorbance was observed with the increasing concentration of all three compounds. The maximum change in absorbance (from 0 to 300 min) for capsaicin, dihydrocapsaicin, and curcumin tests ranged from  $0.33 \pm 0.06$  abs for the control to  $0.09 \pm 0.01$  abs for  $3 \mu\text{M}$  capsaicin,  $0.32 \pm 0.06$  abs for the control to  $0.08 \pm 0.03$  abs for  $3 \mu\text{M}$  dihydrocapsaicin, and  $0.34 \pm 0.07$  abs for the control to  $0.12 \pm 0.04$  abs for  $3 \mu\text{M}$  curcumin. The maximum change in absorbance for all concentrations ( $0.1\text{--}3 \mu\text{M}$ ) of capsaicin was significantly ( $p < 0.03$ ) lower than the control. For dihydrocapsaicin, the minimum concentration to show a significantly lower maximum change in absorbance was  $0.5 \mu\text{M}$ , while for curcumin, it was  $0.7 \mu\text{M}$ . At the highest concentration ( $3 \mu\text{M}$ ) of capsaicin, dihydrocapsaicin, and curcumin, the maximum change in absorbance was 72.8, 75.2, and 65.1% lower than the control, respectively. An increase in lag time (as compared to the control) was observed with increasing concentrations of all three antioxidants. As we were unable to distinguish between the lag phase and the propagation phase at higher concentrations (i.e.,  $2\text{--}3 \mu\text{M}$  for capsaicin,  $1\text{--}3 \mu\text{M}$  for dihydrocapsaicin, and  $3 \mu\text{M}$  for curcumin), the lag time at these concentrations was not determined; however, the lag times at the lowest ( $0.1 \mu\text{M}$ ) concentration of the three compounds were significantly ( $p < 0.001$ ) longer than the respective controls. The lag time increased from  $56.7 \pm 5.7$  min for the control to  $91.7 \pm 7.6$  min at  $1 \mu\text{M}$  capsaicin. Similarly, the lag time increased from  $57.5 \pm 5.8$  min for the control to  $93.2 \pm 9.0$  min at  $0.7 \mu\text{M}$  dihydrocapsaicin, and at  $2 \mu\text{M}$  curcumin, the lag time increased from  $54.0 \pm 2.2$  min (control) to  $168.0 \pm 19.8$  min. The concentrations of capsaicin, dihydrocapsaicin, and curcumin that increased the lag time by 50% were between  $0.5$  and  $0.7 \mu\text{M}$ . Similar to lag time, the rate of oxidation could not be accurately determined at higher concentrations. The rate of oxidation was significantly lower at all concentrations of capsaicin and dihydrocapsaicin, whereas for curcumin, the lowest concentration required to produce a significant reduction in the rate of oxidation was  $0.5 \mu\text{M}$ . At a concentration of  $0.7 \mu\text{M}$ , the rate of oxidation was reduced by 42, 45, and 14% for capsaicin, dihydrocapsaicin, and curcumin, respectively. No significant effects of age and gender were apparent on the kinetic profiles with the three antioxidants.

## DISCUSSION

This study shows that capsaicin, dihydrocapsaicin, and curcumin increase lag time and decrease total in vitro oxidation of serum lipoproteins at concentrations ranging from  $0.1$  to  $3 \mu\text{M}$ . Although earlier studies have reported the effects of chili extracts/capsaicin and curcumin on LDL oxidation (4, 5, 13), to our knowledge, this is the first study to investigate the effects of these compounds on whole serum, which more closely reflects the in vivo situation than isolated LDL. The presence of methoxylated phenol in the structure of capsaicin, dihydrocapsaicin, and curcumin may be responsible for the antioxidative action through its radical-trapping ability as a chain-breaking antioxidant. Although Murakami et al. (4) reported that a lower concentration of capsaicin than dihydrocapsaicin resulted in the same reduction in peroxidation (as measured by thiobarbituric acid reactive substances), we did not observe this difference. The concentration of capsaicin and dihydrocapsaicin that increased the lag time by 50% was similar ( $\sim 0.6 \mu\text{M}$ ). The differences in the results from the two studies are probably due to the differences in the medium tested and assay used to study

oxidation. For example, the present study examined the effect of copper-induced oxidation on whole serum lipids at 245 nm, and Murakami et al. (4) investigated the effects of iron-induced oxidation on liver microsome lipids at 234 nm.

Earlier studies have reported a reduction in serum lipid peroxides by 33% with regular consumption of 500 mg/day of curcumin (95% purity) for 7 days in healthy human volunteers (14). We have also recently reported that 4 weeks of regular consumption of 30 g/day chili blend (55% cayenne chili; capsaicin content, 33 mg) reduces the rate of copper-induced oxidation ( $\sim 10.5\%$ ) in the serum of men and women (15). In addition, women but not men demonstrated an increase ( $\sim 14\%$ ) in the lag phase after the chili diet. We assumed that this difference was probably due to a higher intake of chili/capsaicin per kg body weight in women as compared to men (mean  $0.4 \text{ mg/day/kg}$  body weight as compared to  $0.5 \text{ mg/day/kg}$  body weight). The results of the present study confirm our assumption, since the effects of capsaicin and dihydrocapsaicin on serum lipid oxidation were found to be concentration-dependent. The results of the above-mentioned ex vivo and the present in vitro study suggest that a relatively small amount of chili may be required to produce a modest 10–15% change in overall oxidation.

Although spices such as chili and turmeric have traditionally been an integral part of Asian cuisine, their use in Western cooking has increased dramatically over the last 2–3 decades. Because such “spices” provide some antioxidant activity and hence may have some implications for reducing the risk of coronary heart disease, further research is warranted to evaluate the minimum amounts of combinations of these spices (as often used together in curries) required to be consumed for a modest reduction in serum lipid oxidation.

## ABBREVIATIONS USED

ANOVA, analysis of variance; GLM, general linear modeling; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

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