Chapter 5

Effect of capsaicin, dihydrocapsaicin and curcumin on the copper-induced oxidation of human serum lipids in vitro

5.1 Abstract

Objective: Oxidation of LDL is believed to be the initiating factor for the development and progression of atherosclerosis. The active ingredients of spices such as chilli 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 than using isolated LDL.

Design: We investigated the effects of different concentrations (0.1 to 3µM) of capsaicin, dihydrocapsaicin and curcumin on copper-induced oxidation of serum lipoproteins. Lag time (before initiation of oxidation) and rate of oxidation (slope of propagation phase) were calculated.

Results: Lag time increased and rate of oxidation decreased with the increasing concentrations of the tested antioxidants. A 50% increase in lag time (from control) was observed at concentrations between 0.5 to 0.7µM for capsaicin, dihydrocapsaicin and curcumin. Although the rate of oxidation decreased with increasing concentrations of the tested antioxidants, the overall oxidation was lower with capsaicin and dihydrocapsaicin compared to curcumin.

Conclusion: This study shows that oxidation of serum lipids is reduced by capsaicinoids and curcumin in a concentration-dependent manner.

Footnote: The material in this chapter has been published (with minor editing) as "Effects of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of human serum lipids" in the Journal of Agricultural and Food Chemistry (471).
5.2 Introduction

Oxidation of LDL is believed to contribute to the development and progression of atherosclerosis. Highly cytotoxic oxidised low density lipoprotein (LDL) induces changes in endothelial cells, and enhances proliferation of monocytes and smooth muscle cells (155, 319). Chilli extracts, capsaicin (the active ingredient of chilli), 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 (391-393). Similarly, curcumin the active ingredient of turmeric has been shown to increase the resistance of LDL to oxidation (393). Capsaicin, dihydrocapsaicin and curcumin are fat soluble compounds (359). In rats the absorption of the capsaicinoids has been found to be about 60 to 80% (364), while that of curcumin be about 13%-60% (472, 473).

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 anti-aggregatory properties (14) 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.
5.3 Materials and Methods

Fasting venous blood samples from six healthy individuals (3 men and 3 women, mean age 34 ± 10 years), collected in tubes without anticoagulant, were allowed to clot in the dark at room temperature, 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. (432). 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 3µM) of capsaicin (purity ≥ 98.5%; Tocris, USA), dihydrocapsaicin (purity ≥ 90%; Fluka, Switzerland) and curcumin (purity ≥ 95%; Fluka, Switzerland), and subjected to copper (100µM) induced oxidation. Oxidation kinetics were determined for each serum sample in duplicate by measuring absorbance at 245nm at 37°C using a multi-position 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 antioxidants. Absorbance data was plotted against time. Lag time, an indicator of the resistance of the serum lipoproteins to oxidation, was calculated as the intercept between baseline (time zero) and the tangent of the absorbance curve during the propagation phase. Rate of oxidation was calculated as the slope of propagation phase. Maximum change in absorbance was calculated as the absorbance difference between time zero and time 300 minutes. Freeze-thawing of serum may affect the natural defence of the serum. However our studies have shown that freezing for up to twelve weeks at -80°C has no significant effect in the kinetic profile of oxidation compared with fresh serum (data not shown). Similar results were reported by Schnitzer et al.(474) for serum stored at -15°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 measures ANOVA using general linear modelling (STATA version 8.2, StataCorp LP, USA) was used to test for any differences between the controls and individual concentrations of capsaicin, dihydrocapsaicin and curcumin. Due to the small sample size, the results were also confirmed with OLOGIT analysis, the statistical test used for non-parametric data. The data is presented as mean ± SD unless otherwise reported.

5.4 Results

Oxidation curves for serum incubated with increasing concentrations of capsaicin, dihydrocapsaicin and curcumin are shown in Figure 5.1. A decrease in overall absorbance was observed with the increasing concentration of all three compounds. The maximum change in absorbance (from zero min to 300 min) for capsaicin, dihydrocapsaicin and curcumin tests ranged from 0.33 ± 0.06 abs for control to 0.09 ± 0.01 abs for 3µM capsaicin, 0.32 ± 0.06 abs for control to 0.08 ± 0.03 abs for 3µM dihydrocapsaicin and 0.34 ± 0.07 abs for control to 0.12 ± 0.04 abs for 3µM curcumin. The maximum change in absorbption for all concentrations (0.1 to 3 µM) of capsaicin was significantly (p < 0.03) lower than the control. For dihydrocapsaicin, the minimum concentration to show a significantly lower maximum change in absorbption was 0.5 µM, while for curcumin, it was 0.7 µM. At the highest concentration (3µM) of capsaicin, dihydrocapsaicin and curcumin, the maximum change in absorbption was 72.8%, 75.2% and 65.1% lower than the control, respectively. An increase in lag time (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. 2-3 µM for capsaicin, 1-3 µM for
dihydrocapsaicin and 3 µM for curcumin), the lag time at these concentrations was not
determined, however, the lag times at the lowest (0.1 µM) concentration of the three
compounds were significantly (p<0.001) longer than the respective controls. The lag
time increased from 56.7 ± 5.7 min for control to 91.7 ± 7.6 min at 1µM capsaicin.
Similarly, the lag time increased from 57.5 ± 5.8 min for control to 93.2 ± 9.0 min at
0.7µM dihydrocapsaicin, and at 2µM curcumin, the lag time increased from 54.0 ± 2.2
min (control) to 168.0 ± 19.8 min. The concentrations of capsaicin, dihydrocapsaicin
and curcumin that increased the lag time by 50% were between 0.5 and 0.7 µM. 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 µM. At a
concentration of 0.7 µM, 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.
Figure 5.1 Copper-induced oxidation curves for serum with different concentrations of capsaicin, dihydrocapsaicin and curcumin. Values are shown as mean ± SEM for six duplicate determinations with each concentration of capsaicin, dihydrocapsaicin and curcumin.
5.5 Discussion

This study shows that capsaicin, dihydrocapsaicin and curcumin increase lag time and decrease total \textit{in vitro} oxidation of serum lipoproteins at concentrations ranging from 0.1µM to 3µM. Although earlier studies have reported the effects of chilli extracts/capsaicin and curcumin on LDL oxidation (392-394), to our knowledge this is the first study to study the effects of these compounds on whole serum which more closely reflects the \textit{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. (392) reported that a lower concentration of capsaicin than dihydrocapsaicin resulted in the same reduction in peroxidation (as measured by thiobarbituric acid-reactive substances TBARS), we did not observe this difference. The concentration of capsaicin and dihydrocapsaicin that increased the lag time by 50% was similar (~0.6µM). 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 245nm, Murakami et. al.(392) investigated the effects of iron-induced oxidation on liver microsome lipids at 234nm.

Earlier studies have reported a reduction in serum lipid peroxides by 33% with regular consumption of 500mg/day of curcumin (95% purity) for seven days in healthy human volunteers (475). We have also recently reported that four weeks of regular consumption of 30g/day chilli blend (55% cayenne chilli, capsaicin content 33 mg) reduces the rate of copper-induced oxidation (~10.5%) in the serum of men and women (461). In addition, women but not men demonstrated an increase (~14%) in the lag phase after the chilli diet. We assumed that this difference was probably due to a higher intake of chilli/capsaicin per kg body weight in women compared to men (mean
0.4mg/day/kg body weight compared to 0.5mg/day/kg body weight). The results of the present study support 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 chilli may be required to produce a modest 10-15% change in overall oxidation.

Although spices such as chilli and turmeric have traditionally been an integral part of Asian cuisine, their use in Western cooking has increased dramatically over the last two to three decades. Since 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.

Footnote: In addition to examining the effects of capsaicin, dihydrocapsaicin and curcumin, experiments were also conducted to assess the incubation effects of different concentrations of piprine (active ingredient of black pepper) and lycopene (the colour pigment of tomatoes) on *in vitro* whole serum copper-induced oxidation. However, these experiments were not successful for the following reasons. There was no significant difference in the oxidation curves from control (0 µM) to 20 µM concentration of piprine, after which there was too much of ‘noise’ (due to precipitation) and the oxidation curves were not clear. Pure lycopene is soluble in hexane, tetrahydrofuran or chloroform. All these solvents were not appropriate for the oxidation test, as hexane does not mix with phosphate buffer (the serum diluting agent used in the study) and tetrahydrofuran and chloroform are incompatible with oxidizing agents. Finally, as lycopene is sensitive to light and oxygen, the manufacturer’s instructions were to dissolve lycopene (in any of the above stated solvents) under nitrogen, at zero degrees Celsius in the dark!!