

Short Communication

Effects of daily ingestion of chilli on serum lipoprotein oxidation in adult men and women

Kiran D. K. Ahuja and Madeleine J. Ball*

School of Human Life Sciences, University of Tasmania, Launceston, Tasmania 7250, Australia

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Laboratory studies have shown that the resistance of isolated LDL-cholesterol or linoleic acid to oxidation is increased in incubations with chilli extracts or capsaicin – the active ingredient of chilli. It is unknown if these *in vitro* antioxidative effects also occur in the serum of individuals eating chilli regularly. The present study investigated the effects of regular consumption of chilli on *in vitro* serum lipoprotein oxidation and total antioxidant status (TAS) in healthy adult men and women. In a randomised cross-over study, twenty-seven participants (thirteen men and fourteen women) ate ‘freshly chopped chilli’ blend (30 g/d; 55 % cayenne chilli) and no chilli (bland) diets, for 4 weeks each. Use of other spices, such as cinnamon, ginger, garlic and mustard, was restricted to minimum amounts. At the end of each dietary period serum samples were analysed for lipids, lipoproteins, TAS and Cu-induced lipoprotein oxidation. Lag time (before initiation of oxidation) and rate of oxidation (slope of propagation phase) were calculated. There was no difference in the serum lipid, lipoproteins and TAS at the end of the two dietary periods. In the whole group, the rate of oxidation was significantly lower (mean difference $-0.23 \text{ absorbance} \times 10^{-3}/\text{min}$; $P=0.04$) after the chilli diet, compared with the bland diet. In women, lag time was higher (mean difference 9.61 min; $P<0.001$) after the chilli diet, compared with the bland diet. In conclusion, regular consumption of chilli for 4 weeks increases the resistance of serum lipoproteins to oxidation.

Diet: Intervention: Chilli: Capsaicin: Antioxidants

Traditional medicine has long recognised the value of flavouring agents, herbs and spices. Herbalists have valued chilli, the pungent red pepper used extensively in South American and Asian cuisine, for its various therapeutic actions including anti-inflammatory, and antiseptic effects (Dasgupta & Fowler, 1997). Capsaicin, the active ingredient of chilli, has been used topically for treatment of cutaneous allergy, and neurological disorders such as diabetic neuropathy (Palevitch & Craker, 1995).

Chilli and capsaicin have been found to increase energy expenditure (Kawada *et al.* 1986a; Yoshioka *et al.* 1998), carbohydrate oxidation (Lim *et al.* 1997) and reduce adipose tissue (Kawada *et al.* 1986b). Capsaicin also suppresses bacterial growth and inhibits platelet aggregation (Cichewicz & Thorpe, 1996). Capsaicin decreases the oxidative stress, measured as malondialdehyde, in the liver, lung, kidney and muscle (Lee *et al.* 2003) and reduces plasmid DNA oxidation. Chilli extracts, capsaicin and other capsaicinoids (such as capsate and dihydrocapsate), when incubated with isolated LDL cholesterol and/or oils and fats increase their resistance to oxidation, by delaying the initiation of oxidation and/or slowing the rate of oxidation (Murakami *et al.* 2001; Naidu & Thippeswamy, 2002; Salleh *et al.*

2002). The antioxidative capacity of chilli is higher than ginger, garlic, mint and onion (Shobana & Naidu, 2000). Chillies are also a good source of flavonoids, phenolic acids, carotenoids such as β -carotene and lutein, vitamin A, vitamin C and tocopherols (Palevitch & Craker, 1995), components known for their antioxidative properties (Benzie, 2003).

Oxidation of LDL is hypothesised as the cause for the development and progression of atherosclerosis. Highly cytotoxic oxidised LDL induces changes in the endothelial cells, and enhances proliferation of monocytes and smooth muscle cells (Esterbauer *et al.* 1992; Diaz *et al.* 1997). A number of techniques are available to measure the oxidative stress. Some methods measure the different peroxidation products, such as malondialdehyde, F2-isoprostanes, lipid hydroperoxides, conjugated dienes, glutathione and protein carbonyls, while others test for the oxidation of LDL at different stages (Dotan *et al.* 2004). A commonly used procedure is measuring the resistance of LDL to *in vitro* oxidation by determination of lag time for conjugated diene formation. Using LDL has limitations as an indicator of *in vivo* resistance to oxidation because most of the serum water-soluble antioxidants and pro-oxidants are not present, and these may play an important

Abbreviation: TAS, total antioxidant status.

* **Corresponding author:** Professor Madeleine Ball, fax +61 3 6324 3658, email Madeline.Ball@utas.edu.au

role in resisting or augmenting the oxidation process. Similarly HDL is excluded and this has antioxidative, anti-inflammatory, and anti-aggregatory activities (Barter *et al.* 2004). The use of serum thus provides a better representation of the *in vivo* situation.

We conducted a study to test the hypothesis that regular ingestion of chilli for 4 weeks would increase the resistance of serum lipoproteins to oxidation.

Methods

Men and women aged 22 to 70 years were recruited via newspaper advertisements. Exclusion criteria included: mineral or vitamin supplements; smoking; history of diabetes; heart, hepatic or renal disease. All subjects gave written informed consent. This research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and was approved by the Northern Human Medical and Research Ethics Committee of Tasmania, Australia (application no. 7437).

Experimental design

A randomised cross-over study design was used, with participants allotted to commence a chilli or a no-chilli (bland) diet for 4 weeks, after which they swapped to the second diet. On the chilli diet, participants included 30 g 'freshly chopped chilli' blend per d (MasterFoods™, Wyong, NSW, Australia) in their usual diet. The ingredient composition of the 'freshly chopped chilli' blend was 55% cayenne chilli, water, sugar, salt, acetic acid, xanthan and the nutrient composition per 100 g was: energy, 354 kJ; protein, 1.7 g; fat, 1.2 g; total carbohydrate, 20.7 g; sugar, 14.7 g; Na, 1127 mg. The bland diet was the participants' usual diet without any chilli. Participants were instructed to use minimum quantities of spices, such as cinnamon, garlic and turmeric, on both diets. The diets were iso-energetic to the participants' usual diets with the aim of weight maintenance and participants were asked to keep their physical activity similar on the two dietary periods. Participants completed a 4 d weighed food record in the last week of each diet, which was analysed (FoodWorks version 3.2; Xyris Software, Brisbane, Australia) to compare the macronutrient intake on the two diets. On day 29 of each dietary period, body weight was measured, fasting venous blood samples were collected in vacutainers (without anticoagulant) and allowed to clot in the dark at room temperature, then centrifuged at 1335 g at 4°C for 20 min. The separated serum was frozen at -80°C for later analysis of serum lipoprotein oxidation and total antioxidant status.

Serum lipids, lipoproteins and total antioxidant status (TAS) were analysed, using DataPro clinical analyser (Thermo Electron Corporation, Melbourne, Australia). Serum total cholesterol, HDL and triacylglycerols were measured using enzymic reagents (ThermoTrace, Melbourne, Australia) and LDL was calculated. TAS was measured using a two-reagent Total Antioxidant Status kit (Randox Laboratories Ltd, Crumlin, County Antrim, UK) at 600 nm wavelength. Cu-induced oxidation of serum was undertaken using the method described by Schnitzer *et al.* (1998). To reduce inter-assay variability, samples from the same subject were analysed in the same run. Briefly, serum was thawed

and diluted 50-fold in PBS (pH 7.4). Oxidation was initiated with 100 µM-Cu. Oxidation kinetics were determined in duplicate by measuring absorbance at 245 nm at 37°C using a multi-position spectroscope (Cary-Varian 1E; Varian, Palo Alto, CA, USA) with Cary WinUV software version 1.00 (Varian) every 10 min for 400 min. Absorbance was then plotted against time. Lag time, an indicator of protection of the lipoproteins against 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. To avoid any investigator bias, a blinded observer calculated the lag phase and slope of the propagation phase. The intra-assay CV for lag time was 4.5%.

Statistics

Data were analysed using STATA (version 8.2) software (StataCorp LP, College Station, TX, USA). ANOVA using the general linear model was performed to analyse the differences in serum lipoprotein oxidation and TAS on the two diets, adjusting for order and period effect. Results are presented as means, mean differences, 95% CI and *P* value.

Results

Fourteen men and thirteen women aged 46 (SD 12) years, weight 76.96 (SD 16.72) kg and BMI 26.57 (SD 4.83) kg/m² participated in the study. There was no significant order or period effect on any of the measured parameters. Dietary intake of macronutrients, fibre, vitamin C and alcohol was similar between the two diets. Table 1 shows the comparisons for serum lipids, lipoproteins, lag time, rate of oxidation and TAS at the end of the bland and chilli diets. The rate of oxidation was lower by 10.4% (*P*=0.04) on the chilli diet compared with the bland diet. Table 2 presents the results for body weight and serum oxidation parameters, separated by sex. Compared with the bland diet, women had a longer lag time by 13.3% (*P*<0.001) on the chilli diet. In men, the rate of oxidation was lower by 12.6% (*P*=0.05) on the chilli diet.

Discussion

The data suggest that chilli, eaten regularly for 4 weeks as flavouring, helps inhibit the oxidation of serum lipoproteins, by reducing the rate and susceptibility to oxidation. To our knowledge the present study is the first human study to test this. The longer lag phase in women, but not in men, after 4 weeks of regular chilli consumption may have been due to the higher amount of chilli or capsaicin available per kg body weight. These findings may be of significance to the risk of lipoprotein oxidation in the arterial intima. Foods such as garlic, eaten in small amounts (as is chilli), have been shown to increase resistance to LDL oxidation (Munday *et al.* 1999) and *in vivo* lipid oxidation measured as plasma and urine isoprostanes (Dillon *et al.* 2002).

The observed results may have been due to the capsaicin (the active ingredient of chilli), or a combined effect with other antioxidant vitamins such as vitamin C and carotenoids,

Table 1. Serum lipids, lipoproteins, parameters of serum oxidation and total antioxidant status at the end of the bland and chilli diets (*n* 27)
(Mean values and 95 % confidence intervals)

Variable	Mean	95 % CI	MD	95 % CI	<i>P</i>
Total cholesterol (mmol/l)					
Bland	5.75	5.39, 6.10			
Chilli	5.67	5.27, 6.10	−0.06	−0.26, 0.14	0.56
LDL (mmol/l)					
Bland	3.94	3.64, 4.25			
Chilli	3.87	3.54, 4.19	−0.07	−0.23, 0.85	0.37
HDL (mmol/l)					
Bland	1.42	1.24, 1.61			
Chilli	1.42	1.22, 1.61	−0.002	−0.06, 0.05	0.93
Triacylglycerols (mmol/l)					
Bland	1.70	1.25, 2.16			
Chilli	1.75	1.26, 2.27	0.05	−0.13, 0.25	0.33
Lag time (min)					
Bland	68.70	60.32, 77.09			
Chilli	71.81	64.32, 79.31	2.58	−3.58, 8.73	0.41
Rate of oxidation (absorbance × 10 ^{−3} /min)					
Bland	2.45	2.16, 2.74			
Chilli	2.22*	2.00, 2.45	−0.23	−0.44, −0.01	0.04
Total antioxidant status (mmol/l)					
Bland	1.02	0.93, 1.12			
Chilli	1.05	0.95, 1.15	0.03	−0.02, 0.07	0.28

MD, mean difference after adjusting for order and period effects of diets.

* Mean value was significantly different from that for the bland diet.

Table 2. Body weight and parameters of serum lipoprotein oxidation at end of the bland and chilli diets, by sex
(Mean values and 95 % confidence intervals)

Variable	Men (<i>n</i> 13)					Women (<i>n</i> 14)				
	Mean	95 % CI	MD	95 % CI	<i>P</i>	Mean	95 % CI	MD	95 % CI	<i>P</i>
Weight (kg)										
Bland	85.40	76.77, 93.99				68.92*	59.96, 77.88			
Chilli	85.60	76.82, 94.38	0.27	−0.25, 0.78	0.31	69.16*	60.30, 78.03	0.40	−0.21, 1.00	0.20
Lag time (min)										
Bland	74.38	60.11, 88.66				63.43	53.08, 73.78			
Chilli	71.54	57.79, 85.29	−2.48	−10.97, 6.02	0.57	72.07†	63.13, 81.01	9.61	3.06, 16.16	<0.001
Rate of oxidation (absorbance × 10 ^{−3} /min)										
Bland	2.58	2.08, 3.08				2.33	1.97, 2.70			
Chilli	2.30†	1.91, 2.69	−0.29	−0.58, 0.00	0.05	2.15	1.85, 2.45	−0.19	−0.49, 0.11	0.21

MD, mean difference after adjusting for order and period effects of diets.

* Mean values were significantly different from those of the men (*P*=0.004).

† Mean values were significantly different from those for the bland diet.

including β -carotene, lutein and some tocopherols present in the chilli. Intervention and meta-analysis studies have shown that naturally occurring antioxidants, especially vitamin C, β -carotene and vitamin E consumed in food as part of a healthy diet, are more beneficial than when taken as pure individual supplements (Vivekananthan *et al.* 2003; Hasnain & Mooradian, 2004; Blomhoff, 2005). Combining effects may be beneficial because 'antioxidants with different chemical properties may recharge each other in an antioxidant network' (Blomhoff, 2005).

The difference in mechanism of action between TAS and the Cu-induced serum lipoprotein oxidation assay may be the reason for not observing a difference in TAS between the bland and the

chilli diets. While TAS measures radical-scavenging activity in the aqueous system, the phenolic antioxidants inhibit serum and LDL oxidation not only by the scavenging of free radicals, but also by scavenging reactive oxygen species, metal chelation, protecting α -tocopherol present in LDL and binding with proteins (Frankel & Meyer, 2000).

In conclusion, the results of the study suggest that regular consumption of moderate amounts of chilli, in addition to providing flavour, may help in resisting lipoprotein oxidation. Further studies are warranted, especially with varied amounts of chilli, to investigate the minimum amount of chilli required that is beneficial and more acceptable to a wider section of the population.

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