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Abstracts and posters from conference presentations

SERUM CONCENTRATION OF CAROTENOIDS IN HEALTHY ADULTS ON VARIOUS CAROTENOID CONTROLLED DIETS

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Introduction: It is thought that the absorption and serum levels of fat-soluble antioxidants and carotenoids like lycopene, β-carotene, α-carotene, β-cryptoxanthin and lutein may depend on the amount and type of fat present in the diet as well carotenoid content. However little data is available to support this suggestion. We investigated the effects of a high and a low fat diet on serum carotenoid concentrations, especially lycopene.

Methods: Two separate randomized crossover dietary intervention studies were undertaken. The participants included healthy volunteers aged 22 to 70 years (n = 18 study one, n = 21 study two). The dietary periods comprised a high monounsaturated fat diet (MUFA) and a high carbohydrate low fat (HCLF) diet for 16 and 10 days, on study 1 and study 2, respectively. The diets were controlled for other macronutrients, and were high in lycopene but low in other carotenoids. Dietary lycopene was lower in study 1 (~15mg/day) than study 2 (20mg/day). Fasting blood samples obtained at commencement and end of each dietary period were analysed for serum carotenoids using HPLC.

Results: For each study, serum carotenoids changed similarly on the two (MUFA and HCLF) diets. Serum β-cryptoxanthine and α-carotene concentrations fell (p<0.05). In study 1, serum lycopene increased on the MUFA diet (p < 0.05); however there was no difference at the end of the two dietary periods. In study 2, serum lycopene increased (p < 0.01) on both the diets to similar and higher levels.

Conclusion: These results suggest that, at least for short terms (10-16 days), serum carotenoid concentration is dependent on dietary carotenoid intake, and is not influenced by a change of dietary fat from 15% - 38% of energy.

Oral presentation by K Ahuja at the Annual International Conference of the Society for Free Radical Research (2005), Bangalore, India.
**Effects of olive oil and tomato lycopene combination on heart disease risk factors**

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**Background:** There remains debate about the relative benefits of high monounsaturated fat diets or high carbohydrate diets in reducing the risk of coronary heart disease. Intake of lycopene from tomatoes and tomato products has been suggested as inversely related to the risk of coronary heart disease and some forms of cancer. However little is known about the effects of combination of olive oil and lycopene on the risk factors of heart disease.

**Objective:** To compare the effect of two diets (a monounsaturated fat enriched olive oil diet and high carbohydrate low olive oil diet), with controlled carotenoid content on serum lycopene, lipids and susceptibility of serum to *in vitro* oxidation.

**Design:** A randomised crossover dietary intervention study, in human subjects (20-70 years), of two dietary periods (olive oil enriched, and high carbohydrate low olive oil) of 10 days duration. Both the diets were matched for basic foods and were controlled for carotenoid content, which was high in lycopene.

**Results:** Both diets similarly increased serum lycopene levels. Serum high density lipoprotein cholesterol levels were higher; and triglycerides and low density lipoprotein to high density lipoprotein ratio were lower at the end of the high olive oil diet compared to the high carbohydrate low olive oil diet. No difference was seen in susceptibility of serum to *in vitro* oxidation between the two diets.

**Conclusion:** A high olive oil diet with high lycopene content may reduce the risk of coronary heart disease by increasing serum lycopene levels and improving serum lipid profile.

**Sponsorship:** The study was funded by the Clifford Craig Medical Research Trust, Launceston, Tasmania, Australia. H.J. Heinz, Melbourne, Australia and IGA Moonah, Tasmania, Australia respectively provided the tomato products and olive oil. Jane Pittaway kindly assisted with technical aspects.

_Oral presentation by K Ahuja at the Annual meeting of Nutrition Society of Australia (2003), Hobart, Australia._
EX VIVO AND IN VITRO ACTIONS OF CHILLI AND CAPSAICIN ON DNA OXIDATION
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Introduction: The fruit of Capsicum frutescens, ‘chilli pepper’, contains several antioxidants, including the active ‘pungent’ capsaicinoids, capsaicin and dihydrocapsaicin. The aims of the present study were to determine whether (a) ingestion of chilli protects mononuclear cell DNA against hydrogen peroxide (H$_2$O$_2$)-induced oxidation ex vivo and (b) capsaicin protects mononuclear cell DNA against hydrogen peroxide (H$_2$O$_2$)-induced oxidation in vitro.

Methods: Using a randomized cross-over design, eleven healthy subjects (mean±SD: age, 42.0±12.4 yr; weight, 78.6±14.6 kg; body mass index, 27.7±4.7; M:F, 3:8) consumed a chilli-free diet (normal diet with no chilli and minimal black pepper, ginger, etc) or chilli-supplemented diet (normal diet plus 30g/day MasterFoods™ crushed chilli) for four weeks. Mononuclear cells were isolated from whole blood at the end of each diet by density gradient centrifugation over histopaque, frozen (Nalgene Cryo) and stored at -70°C (Duthie et al. 2002). The resulting cell population was lymphocyte-enriched (~85%). The resistance of mononuclear cell DNA to oxidation by H$_2$O$_2$ ex vivo was assessed using the comet (single cell electrophoresis) assay. One hundred comets on duplicate slides were scored [1 (no damage) to 4 (maximal damage)] and averaged.

Results: Following the chilli diet, H$_2$O$_2$–induced DNA strand breakage was lower in 9 of the 11 subjects and unchanged in two subjects compared with the chilli-free diet. Mean (±SEM) H$_2$O$_2$–induced DNA strand breakage was significantly lower ($P<0.005$, paired t-test) after the chilli diet (113±20) compared with the chilli-free diet (147±20). The effect of capsaicin in vitro on oxidation of mononuclear cell DNA by H$_2$O$_2$ was also assessed by maintaining human mononuclear cells (from fasting subjects) for two hours in capsaicin (0.5–100µM) or the antioxidant, quercetin (50 µM). Quercetin but not capsaicin protected against H$_2$O$_2$-induced DNA breakage.

Conclusion: This study shows that intake of chilli peppers on a regular basis provides protection against oxidative damage to human mononuclear cell DNA. The antioxidant protection provided by chilli ingestion probably results from the action of several constituents (phytochemicals) found in chilli fruit, rather than capsaicin alone.


Oral presentation by M. Garvey at the Joint meeting of Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists and Australasian Pharmaceutical Science Association (2005), Melbourne, Australia.
Lycopene and Olive oil Combination
A step towards reducing CHD risk?

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Background: After years of research it is still debatable which energy source (carbohydrates or unsaturated fats) should replace the dietary saturated fats in order to reduce the risk of coronary heart disease (CHD). Recent research on carotenoids, fat soluble compounds found mainly in fruits and vegetables, proposes that populations with high dietary/serum levels of carotenoids, including lycopene (found in tomatoes), have lower risk of CHD. However it is still unclear whether fat in the diet can affect the absorption of carotenoids leading to one combination being preferable to another.

Aim: To compare the effects of lycopene enriched diets, one high in monounsaturated fat from olive oil and the other with low fat, high carbohydrate content on the serum lycopene and CHD risk factors.

Method: Thirteen healthy female subjects aged 25-55 years were recruited and randomly assigned to commence on either an olive oil (OO) diet or a low fat high carbohydrate (LF) diet, after a two day low carotenoid diet (LCD). The LCD was to avoid any peaks in serum carotenoids that may occur 12-14 hours after a carotenoid rich meal.

The OO and LF diets were isocaloric and contained the same basic foods. The diets were high in lycopene and low in other carotenoids. Main sources of lycopene in the diet were tomato soup and tomato paste. Each diet was of ten days duration with an 18 days “washout” period between. The OO diet was designed to provide 36 - 38% of energy from fat (mainly olive oil) whereas LF diet contained only 14-16% of energy from fat. Subjects recorded a 4 day diet record towards the end of both diet periods. Fasting blood samples and anthropometric measurements were taken at the start and the end of each diet period. Serum carotenoids, cholesterol and lipoproteins were analysed.

Results: The mean (SD) age of the study group was 41.5 (8.4) years and BMI of 23.2 (2.5). There was no change in body weight after the two dietary periods. The two diets were not significantly different in energy or nutrient content except for dietary fat and carbohydrate. Both the diets produced a significant increase in the serum lycopene and decrease in alpha carotene levels, but no significant difference was noted between the ends of the two dietary periods (Fig.1). Serum total cholesterol and LDL cholesterol decreased significantly after both the OO and the LF dietary periods (Fig. 2), but there was no significant difference at the end of the two diets. A decrease in serum triglycerides and HDL was seen after the OO and the LF diets respectively. HDL cholesterol was higher at the end of the OO diet compared to the LF diet.

Conclusion: This short term intervention study suggests that the amount of fat in the diet does not affect serum lycopene levels, but levels rise on a high lycopene diet. An olive oil containing diet appears to improve the serum lipid profile.

Acknowledgements: This study was funded by Clifford Craig Medical Research Trust, Launceston. Heinz Watties, Melbourne kindly donated tomato products and IGA, Moonah donated olive oil for the study.

Presented as poster as at the Health & Medical Research Week Meeting (2003), Launceston General Hospital, Launceston.
Introduction
The active ingredient of chilli pepper, capsaicin, produces dose-dependent vasoconstriction in experimental animals (Griffiths et al, 1996). Although application of capsaicin locally to the skin induces vasodilation (Munce & Kennedy, 2003), there is no information available on the effects of regular capsaicin consumption (in the form of chilli) on vascular function in humans.

Aim
The aim of the present study was to determine the effects of chilli supplementation on endothelium-independent and endothelium-dependent vasodilatation.

Methods
Fifteen non-smokers (M/F 7/8; aged 47.3 ± 11.8 (±SD) years; weight 78.9 ± 14.2 kg; body mass index 27.3 ± 3.7 kg/m²) with no history of hypercholesterolemia, diabetes mellitus or renal dysfunction consumed a chilli-free bland diet (normal diet with no chilli and minimal black pepper, ginger, etc) or chilli-supplemented diet (normal diet plus 30g/day ‘Freshly chopped chilli’ blend (MasterFoods®, Australia) for three weeks each. After three weeks on each diet, heart rate, fasting peripheral and central (aortic) blood pressure, augmentation pressure (AP), augmentation index (AIX), AIX at HR=75 beats per minute (AIX@HR75) and subendocardial viability ratio (Buckberg index) were determined using a sphygmomanometer and pulse wave analysis (SphygmoCor, AtCorMedical, Australia) (Wilkinson et al, 2002). AIX and subendocardial viability ratio (SEVR) are measures of arterial stiffness and coronary perfusion, respectively.

Results
After adjusting for order and period of diet, mean (±SD) baseline AIX (23.97 ± 10.56) and AIX@HR75 (15.77 ± 8.59) were significantly lower (P < 0.05, general linear model) after the chilli diet compared to the chilli-free diet (AIX, 27.97 ± 10.45; AIX@HR75, 19.40 ± 7.87). All parameters were then measured at regular intervals for 30 min after sublingual GTN (600 µg) and for 20 min after inhaled salbutamol (200 µg). Although AIX and AIX@HR75 at ten and 15 min after GTN was lower on the chilli diet compared to the bland diet, there was no difference in the maximal change in any of the measured parameters or overall vasodilator response (area-under-the-curve) in response to GTN or salbutamol between the two diets.

Conclusion
These results suggest that regular chilli consumption reduces baseline vascular stiffness but has no significant effect on either endothelium-independent or -dependent vasodilatation.


Presented as poster at the joint meeting of Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists and Australasian Pharmaceutical Science Association (2005), Melbourne, Australia.
Effect of meals containing chilli on glucose metabolism and energy expenditure

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Introduction
Meals containing chilli have been shown to increase energy expenditure (EE) and/or decrease energy intake in lean young adults. As habitual diets may affect the activity and responsiveness of receptors involved in regulation and transportation of nutrients, we investigated the metabolic effects of meals containing chilli in adult subjects after two different background diets.

Aim
To investigate the metabolic effects of a meal containing 30g chilli blend following a bland diet and a chilli blend (30g/day ‘freshly chopped chilli’, Masterfoods®, Australia) supplemented diet.

Methods
Thirty-six participants aged 46 ± 12 (mean ± SD) years, BMI 26.3 ± 4.6 kg/m², participated in a randomized crossover intervention study with two dietary periods (chilli and bland) of four weeks each. Postprandial effects (Figure 1) of meals were evaluated with: a bland meal after bland diet (BAB); a chilli meal after bland diet (CAB); and a chilli meal after chilli-containing diet (CAC). The ingredient composition of the chilli blend was 55% cayenne chilli, water, sugar, salt acetic acid and xanthan. The meals were matched for macronutrient composition (2000kJ, 67% carbohydrate, 18% protein and 15% fat). Serum insulin, C-peptide, glucose and EE were measured at fasting and up to 120 minutes postprandially.

Results
The measured variables were not significantly different at baseline across the three meals. For the whole group, glucose area under curve (AUC) was significantly lower (p < 0.05) after the CAB meal compared to the BAB meal (Figure 2). Maximal insulin concentration and AUC for insulin were significantly lower (p < 0.001) after the CAC meal compared to the BAB meal. No significant difference was observed for EE between the three meals.

When data were separated for participants with BMI below and above 25kg/m², subjects with BMI ≥ 25kg/m² exhibited lower (p < 0.001) maximal C-peptide and EE (p < 0.001) and higher (p < 0.001) C-peptide/insulin quotient (an indicator of hepatic insulin clearance) after the CAC meal compared to the BAB meal (Table 1).

Table 1. Comparison of area under the curve (AUC) for some metabolic parameters on the three meals in different BMI groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BAB (n = 7 to 11)</th>
<th>BAB ≥ 25 (n = 20 to 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µIU/ml/min)</td>
<td>51.51 (12.05 to 90.97)*</td>
<td>11.90 (5.01 to 13.57)*</td>
</tr>
<tr>
<td>C-Peptide (µg/ml)</td>
<td>11.90 (5.01 to 13.57)*</td>
<td>12.79 (11.70 to 13.87)*</td>
</tr>
<tr>
<td>C-peptide/iAUC Insulin</td>
<td>65.56 (12.32 to 125.29)</td>
<td>105.30 (64.80 to 140.75)*</td>
</tr>
<tr>
<td>EE (kJ/day)</td>
<td>11.05 (8.09 to 14.45)</td>
<td>12.31 (11.23 to 13.42)</td>
</tr>
<tr>
<td>Maximal C-peptide</td>
<td>11.05 (8.09 to 14.45)</td>
<td>12.31 (11.23 to 13.42)</td>
</tr>
<tr>
<td>Maximal EE</td>
<td>12.31 (11.23 to 13.42)</td>
<td>12.31 (11.23 to 13.42)</td>
</tr>
</tbody>
</table>

Conclusion
Habitual consumption of chilli may help in ameliorating meal-induced hyperinsulinaemia by increasing hepatic insulin clearance, but as a negative effect may also lead to lower thermogenesis in overweight and obese people.

Presented as poster at the Heart Foundation Conference and Scientific meeting (2006), Sydney, Australia.
Chilli Diet May Affect Sleep Quality And Daytime Activity
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Introduction: Spicy meals have been shown to affect sleep quality acutely. In a study of six, young, healthy male subjects, Tabasco sauce and mustard taken with the evening meal reduced slow wave and stage 2 sleep, increasing total time awake and tending to increase sleep onset latency (Edwards et al, 1992). However, there is little information on the longer-term effects of the ingestion of hot principles on sleep quality and daytime activity.

Method: As part of a wider pilot study of the effects of a chilli diet on cardiovascular protective activity, 25 healthy subjects were recruited to study the effects of chilli in the diet on sleep. Subjects were placed on controlled diets for eight weeks, with four weeks in which their diet was free of chilli and other foods containing capsaicin or related compounds and four weeks of the same diet with the addition of 30g of chopped chilli (Masterfoods®, Australia). The order of consumption of the chilli or non-chilli diet was randomized. Sleep quality was assessed subjectively using a modification of the Leeds Sleep Evaluation Questionnaire (LSEQ) in 25 subjects and objectively in 8 subjects using actigraphy (Minimitter, USA) during each diet. The actigraphs recorded the number of movements every 30 seconds over 5 days.

Results: The questionnaire results overall indicated that sleep latency, awakening and daytime function were improved by a chilli diet, with no effect on sleep quality (Figure 1). However, four subjects commented that their daytime activity and alertness was enhanced on chilli diet, and one experienced vivid dreams on chilli. Actigraphy suggests that there was no change in sleep latency, but average actual sleep time was decreased by 20 minutes on chilli and subjects went to bed on average 2 hours ten minutes later when on the chilli diet compared to the non-chilli diet. Sleep fragmentation was not changed, but the total activity during sleep decreased by 19.2% suggesting sleep architecture was changed (Figure 2). Average total activity over the five days of study was increased in five subjects by 10 to 30%, whilst of three subjects, in which a decrease was measured, one only wore the actigraph at night and was exclude from the measure, one was on holiday during the chilli diet and the third had a BMI of 35 which affects metabolic response to capsaicin (Figure 3).

Conclusion: Thus, the results of this preliminary study suggest that chilli in the diet may improve sleep quality, alter sleep architecture and, in addition, improve daytime function and alertness. As this is after acclimatization to chilli, it may not be due to the sympathetic arousal associated with acute chilli ingestion, but may involve effects on central sleep mechanisms. Further investigation on a larger sample is required to answer these questions.


Acknowledgement: The authors wish to thank Masterfoods® Australia for donating the Fresh Chopped Chilli.

Presented as poster at the 18th Annual Scientific meeting of the Australasian Sleep Association (2005), Gold Coast, Queensland, Australia.