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ORIGINAL ARTICLE

The effect of 4-week chilli supplementation on metabolic and arterial function in humans

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Objective: To investigate the effects of regular chilli ingestion on some indicators of metabolic and vascular function.
Design: A randomized cross-over dietary intervention study.
Setting: Launceston, Australia.
Subjects: Healthy free-living individuals.
Intervention: Thirty-six participants (22 women and 14 men), aged 46±12 (mean±s.d.) years; BMI 26.4±4.8 kg/m^2, consumed 30 g/day of a chilli blend (55% cayenne chilli) with their normal diet (chilli diet), and a bland diet (chilli-free) for 4 weeks each. Metabolic and vascular parameters, including plasma glucose, serum lipids and lipoproteins, insulin, basal metabolic rate, blood pressure, heart rate, augmentation index (AIx; an indicator of arterial stiffness), and subendocardial-viability ratio (SEVR; a measure of myocardial perfusion), were measured at the end of each diet. In a sub-study, during week 3 of each dietary period, the vascular responses of 15 subjects to glyceryl-trinitrate (GTN) and salbutamol were also studied.
Results: For the whole group, there were no significant differences between any of the measured parameters when compared at the end of the two dietary periods. When analysed separately, men had a lower resting heart rate ($P=0.02$) and higher SEVR ($P=0.05$) at the end of the chilli diet than the bland diet. In the sub-study, baseline AIx on the chilli diet was lower ($P<0.001$) than on the bland diet, but there was no difference in the effects of GTN and salbutamol between the two diets.
Conclusion: Four weeks of regular chilli consumption has no obvious beneficial or harmful effects on metabolic parameters but may reduce resting heart rate and increase effective myocardial perfusion pressure time in men.
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Keywords: dietary intervention; chilli; capsaicin; metabolic; arterial compliance

Introduction

Risk factors for cardiovascular disease (CVD) include metabolic and vascular factors, such as hypercholesterolemia, hyperglycaemia, hyperinsulinemia, obesity, hypertension and arterial stiffness. One approach to reducing CVD risk is to reduce body weight. Obesity elevates the risk of hyperglycaemia, hyperinsulinemia, and vascular resistance (Eckel *et al*., 2005; Moller and Kaufman, 2005; Sundell, 2005). Decreasing energy input and increasing energy output (e.g., low energy diets and increasing exercise) are the two approaches for weight reduction. Chilli or its active component capsaicin has been shown to increase energy expenditure and/or fat oxidation (in animals and humans) and lower serum lipids (in animals).

In rats, capsaicin increases oxygen consumption (and hence, energy expenditure) and lipid oxidation, probably due to activation of the sympathetic nervous system and the subsequent release of catecholamines (Kawada *et al*., 1986b; Watanabe *et al*., 1987). High-fat diets containing capsaicin lower adipose tissue weight (Kawada *et al*., 1986a), reduce low-density lipoprotein (LDL) cholesterol and increase high-density lipoprotein (HDL), cholesterol and triglycerides (Kawada *et al*., 1986a; Srinivasan and Chandrasekhara 1992; Saito *et al*., 1999; Lee *et al*., 2003; Tani *et al*., 2004). Small amounts of capsaicin, when used topically, induce vasodilatation, but at higher concentrations cause vasoconstriction (Suzuki *et al*., 1998).

Human data suggest that adding chilli to a meal lowers total energy and macronutrient intake (Yoshioka *et al*., 1999; Yoshioka *et al*., 2001; Yoshioka *et al*., 2004; Belza and Jessen,

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2005). Chilli also increases postprandial energy expenditure (EE) (Henry and Emery 1986; Yoshioka *et al*., 1995; Yoshioka *et al*., 1998), fat oxidation in women (Yoshioka *et al*., 1998) and carbohydrate oxidation in men (Yoshioka *et al*., 1995; Lim *et al*., 1997).

To our knowledge, there are only two small to medium-long-term studies that have investigated the effects of regular intake of the active ingredient of chilli (capsaicin) on metabolism in adult humans (Lejeune *et al*., 2003; Belza and Jessen, 2005). One study (Belza and Jessen, 2005) reported a 'tendency' towards increased energy expenditure after 1 week of regular capsaicin intake (0.6 mg/day) and the other (Lejeune *et al*., 2003), reported the beneficial effect of capsaicin (135 mg/day) in sustaining fat oxidation after a period of weight loss. Both studies used pure capsaicin in supplemental form, rather than chilli as a food.

In the present study, using a randomized cross-over design, we examined the effects of 4-week regular consumption of chilli, as a flavouring agent, on some indicators of metabolic and vascular function. These indicators included serum lipids and lipoproteins, insulin, C-peptide, plasma glucose, basal metabolic rate (BMR), blood pressure, augmentation index (AIx, an indicator of arterial stiffness) and subendocardial-viability ratio (SEVR, a measure of effective myocardial perfusion pressure time).

Subjects and methods

Study participants and protocol

Male and female participants aged between 22 and 70 years were invited, through newspaper articles and university newsletters, to take part in the study. The exclusion criteria included: a self-reported history of heart disease; any prescribed medication except for contraception; weight gain or weight reduction regimes during the last 2 months; every day consumption of chilli; smoking; and pregnancy. All subjects gave their written informed consent. The Northern Human Medical and Research Ethics Committee of Tasmania (Australia) approved the study (H7437).

Using a randomization sheet http://www.graphpad.com/quickcalcs/RandMenu.cfm, 38th participants were allotted to commence either a bland (chilli-free) or a chilli diet, for 4 weeks. On completion of the first diet, participants swapped to the other diet for the next 4 weeks. At the start of the first diet and then at the end of each dietary period (bland and chilli), measures were made of a range of metabolic and vascular parameters to compare the effects of the two diets. There was no washout period because the comparison was between chilli and no chilli diets. It has been reported that it takes 4 weeks for blood lipid levels to reflect the effects of altered dietary intake where iso-energetic conditions prevail (Schaefer *et al*., 1995a, b); and chilli has a short half-life. It was thus expected that any potential 'carry over' effects would not remain by the end of 4 weeks of the diets.

Dietary protocol

Both the diets (chilli and bland) were based on each participant's usual diet. However, products containing chilli and pungent foods including: wasabi, horse radish, mustard, turmeric, black and white pepper and spices such as cinnamon and cumin, were excluded from both diets. The participants were instructed to keep the intake of caffeine drinks such as coffee at no more than three cups a day and to maintain the same intake on the bland and the chilli dietary periods. On the chilli diet, participants added 30 g/day of 'Freshly Chopped Chilli' blend (donated by MasterFoods, Australia) to their usual diet. Participants chose whether they consumed all 30 g of chilli blend as part of one meal, or divided it over several meals. Participants were advised to add chilli to the food *after* cooking. Hence, the bland diet was a spice-free form of the participants' normal diet and the chilli diet was the 'hot' form of the same diet.

In all, 30 g of chilli was decided upon after two palatability test sessions, where 10 members of staff (everyday users as well as occasional users) ingested different products and amounts of chilli with bread. In all, 30 g of MasterFood 'Freshly Chopped Chilli' was widely accepted. The composition of this product was 55% cayenne chilli (*Capsicum annum* species), water, sugar, salt, acetic acid and xanthan. The nutrient composition per 100 g of chilli was: energy 354 kJ; protein 1.7 g; fat 1.2 g; total carbohydrate 20.7 g; sugar 14.7 g and sodium 1127 mg. The manufacturer (Masterfoods, Australia) reported that cayenne pepper contains 2000 ppm of capsaicin (email communication dated 11th February 2003). Using this information the capsaicin content of the product used in the present study was approximately 33 mg/30 g of chilli blend (55% chilli). This is comparable to the amount of capsaicin (30 mg) used in earlier studies that investigated the effects of meals containing chilli on energy metabolism in lean young individuals (Yoshioka *et al*., 1995; Lim *et al*., 1997; Yoshioka *et al*., 1998).

To compare the nutrient intake on the two dietary periods, participants completed a 4-day (two weekdays and two weekend days) weighed food record during the last week of each dietary period, which was analysed with Foodworks software (version 3.02, Xyris, Brisbane, Australia). The nutrient composition provided on the jar of chilli was added to the Foodworks database and the results from the two dietary periods were used to compare macro nutrients, fibre and alcohol intake on the two intervention diets, as well as to check dietary compliance.

Protocol on days of testing

Subjects were asked to fast overnight for 10–12 h and refrain from alcohol, fried food and any vigorous exercise for at least 24 h before the test day/time. Upon arrival at the University clinical area, anthropometric measurements were made and blood samples taken in at the fasting state at rest. Participants then remained seated, in an easy chair (semi-reclining position), to rest for 10–15 min after which time

blood pressure and pulse wave analysis (PWA) measurements were taken for analysis of vascular function (including heart rate (HR), AIx and SEVR), followed by measurement of BMR. All the above measurements were taken in the fasting state.

Anthropometric measurements

Body weight was measured using electronic scales, with subjects in light clothing. Height was measured using a stadiometer. Body composition – fat mass, lean mass and body water was measured using a bioelectrical impedance analysis system with a four electrode body fat analyzer (BF-900, Maltron International Ltd, Essex, UK).

Blood variables

Blood samples were collected in an anticoagulant-free tube (for serum lipids, insulin, C-peptide) and a fluoride-oxalate anticoagulant tube (for plasma glucose). The anticoagulant-free tube was allowed to clot in the dark at room temperature and the fluoride-oxalate tube was immediately put on ice. Both the tubes were centrifuged at 1335 g for 20 min at 4°C. Serum and plasma were separated, aliquotted and frozen at −80°C until further analysis. All biochemical analyses of samples from individual participants were performed in the same run, to eliminate inter-assay variability.

Serum lipids, lipoproteins and plasma glucose were analysed, using DataPro clinical analyzer (Thermo Electron Corporation, Melbourne, Australia). Serum total cholesterol, high-density lipoprotein (HDL) and triglycerides (TG) were measured using enzymatic reagents (ThermoTrace, Australia). Low-density lipoprotein (LDL) was calculated using Friedewald equation (Friedewald *et al*., 1972). Serum insulin and C-peptide were measured by radio-immunoassay, using commercially available kits (Diagnostic Systems Laboratories, Sydney, Australia). The intra-assay coefficient of variance for serum lipids, lipoproteins and plasma glucose was <2% and for serum insulin and C-peptide was <6%.

Basal metabolic rate

BMR (while sitting in a semi-recumbent position), after a rest of 15 min, was measured for 15 min by indirect calorimetry, using an open-circuit ventilated hood system (Deltatrac II, Datex Instrumentation Corp., Helenski, Finland). Oxygen consumption (VO_2 ml/min), CO_2 production (VCO_2 ml/min), and energy expenditure using the Weir equation (Weir, 1949) while assuming a fixed protein catabolism, and respiratory quotient results were displayed on a computer screen and data were captured every minute.

Blood pressure and pulse wave analysis

Brachial artery blood pressure was measured with an Omron digital sphygmometer (model T9P, Omron Healthcare Co., Ltd, IL, USA). Pulse wave analysis (PWA) (SphygmoCor; AtCor Medical, Sydney, Australia) was used to examine some of the parameters of vascular function. PWA is a non-invasive method that employs applanation tonometry to record pressure waves from the radial artery, which are then used to generate the aortic arterial waveform, by validated and generalized reverse transfer function (O'Rourke and Gallagher, 1996; Wilkinson *et al*., 1998). From this arterial waveform, aortic blood pressure, augmentation (AG; the pressure difference between the first and second systolic pressure peaks), augmentation index (AIx; AG divided by pulse pressure) and subendocardial viability ratio (SEVR; ratio of area under the curve for diastolic phase and systolic phase as measure of time) are calculated (Figure 1). AIx is an indicator of arterial stiffness (O'Rourke and Gallagher, 1996) and SEVR is a measure of myocardial perfusion (Buckberg *et al*., 1972; Fokkema *et al*., 2005). As augmentation is

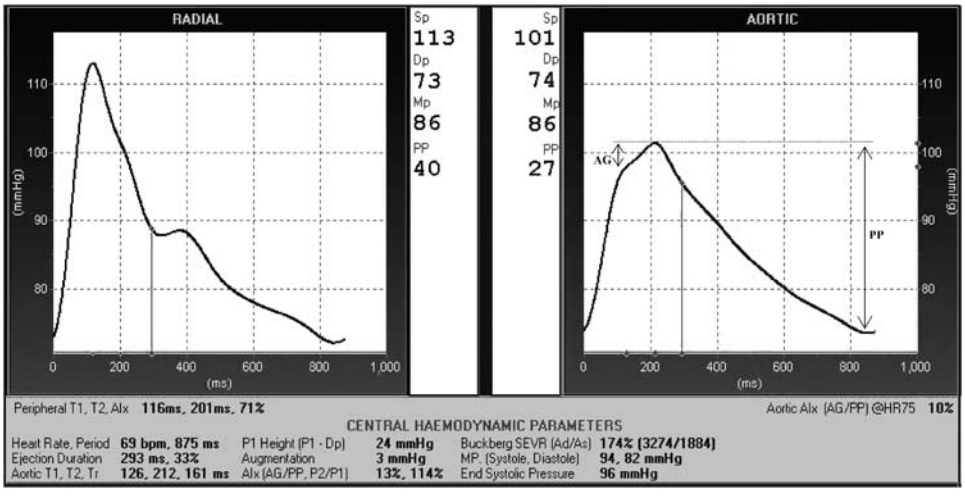

Figure 1 Graphical representation of a radial artery wave form and transferred aortic waveform.

affected by HR (Wilkinson *et al*., 2000), AIx was also normalized to AIx at HR of 75 beat/min (AIx@HR75). All blood pressure and PWA measurements were taken in duplicate, on the same arm, at each time point, by a single observer.

Sub-study

In a sub-study during week 3 of both dietary periods, the vascular responses of 15 participants (seven male and eight female) from the main study to sublingual glyceryl trinitrate (GTN) and inhaled salbutamol were measured to provide a measure of endothelium independent and an endothelium dependent vasodilatation, respectively. GTN is a nitric oxide (a vasodilator) donor whereas salbutamol stimulates the endothelium to synthesize nitric oxide. Previous studies have shown a reduction in pulse pressure and augmentation index (reduced arterial stiffness) after the administration of these drugs, when tested with PWA (Westerbacka *et al*., 2004). Our aim was to investigate if the vascular responses of participants, while on different diets, was similar or different to these two drugs.

Blood pressure and PWA measurements were taken (after 10–12 h of fasting) at baseline and then repeated at 3, 5, 10, 15, 20 and 30 min after the administration of sublingual GTN (600 μg). Salbutamol (200 μg) was then inhaled and blood pressure and PWA measurements were repeated at 5, 10 and 20 min.

Statistical analysis

Statistical analysis was performed using STATA version 8.2, SataCorp LP, USA. Repeated measures ANOVA using the general linear modelling (GLM) with robust standard error estimation analysis (STATA version 8.2, StataCorp LP, USA), after adjusting for order and period effects of diet, was used to test for any differences between the end of the bland and chilli diet. Data for the sub-study was compared at individual time points (while adjusting for order and period of diet). To study the overall change (from fasting to up to 30 min after GTN and then 20 min after salbutamol) the net area-under-the-curve (net-AUC) was calculated by subtracting the negative area (below the baseline) from the positive area (above the baseline) using trapezoidal method (GraphPad PRISM 4, San Diego, CA, USA). This data was then compared using general linear modelling. Results are presented as mean, 95% confidence intervals (CI), mean difference (MD) between chilli and the bland diet, 95% CI of MD and *P*-value, unless otherwise stated. As not all analyses were available for every participant, the number of participants for each variable tested is shown in graphs and tables.

Results

Two participants withdrew from the study, due to work commitments, during their first dietary period (bland). Thirty-six participants (22 female and 14 male) aged 46 ± 12 (mean $\pm$ s.d.) years; body mass index (BMI) $26.4 \pm 4.8\,kg/m^2$ completed the study, of whom 20 commenced the study with the chilli diet and 16 commenced with the bland diet. The measurements at study commencement for plasma glucose, serum total cholesterol, HDL, TG and insulin were 4.92 ± 0.75, 5.89 ± 1.77, 1.54 ± 0.49, 1.68 ± 1.03 mmol/l and 6.40 ± 4.39 μIU/ml, respectively. Measures of the vascular parameters (systolic blood pressure, diastolic blood pressure, AG, HR, SEVR and AIx@HR75) were 115 ± 11, 79 ± 10, 9 ± 4 mm Hg, 63 ± 8 beats/min, 169 ± 24 and 23 ± 11%, respectively.

There was no order or period effect of the diets on any of the measured parameters except for the fasting serum insulin, where a slightly lower result (~ 1.1 μIU/ml) was seen in the second period, irrespective of the order of the diet. No statistically significant change was observed for any of the measured parameters from the start to the end of each dietary period.

There was no statistically significant difference in the dietary intake on the two dietary periods (Table 1). There was no difference in weight, BMI, fat mass, lean mass, predicted

Table 1 Daily energy and macronutrient intake on the bland and the chilli diet ($n = 35$)

Variable	*Bland diet Mean (95% CI)*	*Chilli diet Mean (95% CI)*	*MD (95% CI)*	P
Energy (MJ)	8.5 (7.65–9.27)	8.5 (7.73–9.20)	−0.02 (−0.51–0.47)	0.94
Protein (g)	88.40 (76.10–100.70)	85.32 (73.94–96.71)	−3.51 (−10.86–3.85)	0.35
Fat (g)	74.55 (66.09–83.00)	76.21 (67.50–84.92)	1.09 (−5.24–7.42)	0.74
Carbohydrate (g)	230.10 (204.60–255.60)	232.99 (211.01–254.96)	3.90 (−10.48–18.28)	0.60
Fibre (g)	22.98 (20.10–25.85)	24.42 (18.85–29.99)	1.29 (−3.28–5.86)	0.58
PUFA:SFA	0.39 (0.34–0.45)	0.43 (0.34–0.52)	0.04 (−0.05–0.13)	0.44
Protein (% of energy)	17.73 (15.90–19.56)	17.08 (15.28–18.87)	−0.70 (−1.79–0.39)	0.21
Fat (% of energy)	32.33 (30.42–34.23)	32.75 (30.75–34.75)	0.24 (−1.64–2.11)	0.81
Carbohydrate (% of energy)	45.94 (43.21–48.67)	46.86 (44.06–49.66)	1.28 (−1.02–3.57)	0.28
MUFA (% of fat)	39.29 (38.01–40.57)	39.49 (38.03–40.96)	0.06 (−1.60–1.72)	0.94
PUFA (% of fat)	16.46 (14.88–18.05)	16.89 (15.35–18.42)	0.38 (−1.27–2.03)	0.65
SFA (% of fat)	44.25 (42.03–46.47)	43.62 (40.96–46.27)	−0.44 (−3.20–2.31)	0.75

Abbreviations: CI, confidence interval; MD, mean difference between chilli and bland diet while adjusting for order and period of the diet; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; data compared with general linear model while adjusting for order and period of the diet.

BMR (calculated with Harris-Benedict equation), measured BMR, RQ and VO_2 consumption at the end of the two dietary periods (Table 2). Similarly, plasma glucose, serum lipids, lipoproteins, insulin and C-peptide were not significantly different at the end of the two dietary periods (Table 2). Vascular function parameters (systolic pressure, diastolic pressure, resting heart rate, AG, AIx and SEVR) analysed for whole group were not significantly different. However, men ($n=14$) but not women, on the chilli diet had a significantly lower resting heart rate ($P=0.02$) and a higher SVER ($P=0.05$) compared to the bland diet (Table 3).

Table 2 Anthropometric and some metabolic measurements at the end of the bland and the chilli diet

Variable	N	*Bland diet Mean (95% CI)*	*Chilli diet Mean (95% CI)*	*MD (95% CI)*	P
BMI (kg/m^2)	36	26.30 (24.72–27.88)	26.35 (24.76–27.94)	0.04 (−0.09–0.17)	0.52
Predicted BMR (MJ)	36	6.50 (6.15–6.85)	6.51 (6.16–6.86)	0.01 (−0.01–0.04)	0.19
Measured BMR (MJ)	35	6.42 (5.98–6.86)	6.23 (5.81–6.65)	−0.19 (−0.42–0.03)	0.09
VO_2 (ml/min)	35	225.82 (210.72–290.91)	217.73 (203.27–232.19)	−7.79 (−16.19–0.62)	0.07
RQ (per min)	35	0.81 (0.78–0.83)	0.80 (0.78–0.82)	−0.01 (−0.03–0.02)	0.61
Fat oxidation (g/h)	35	4.36 (3.76–4.97)	4.35 (3.77–4.93)	0.03 (−0.67–0.72)	0.94
Fat mass (%)	35	30.12 (27.58–32.66)	30.05 (27.51–32.59)	−0.11 (−0.86–0.64)	0.78
Fat mass (kg)	35	23.50 (20.48–26.53)	23.47 (20.45–26.50)	−0.07 (−0.72–0.58)	0.84
Lean mass (%)	35	69.94 (67.38–72.49)	69.94 (67.41–72.48)	0.04 (−0.69–0.78)	0.91
Lean mass (kg)	35	52.79 (49.60–55.98)	53.00 (49.67–56.32)	0.23 (−0.34–0.80)	0.42
Glucose (mmol/L)	36	4.93 (4.73–5.13)	4.83 (4.57–5.10)	−0.10 (−0.26–0.05)	0.19
Insulin (μIU/ml)	34	5.48 (4.19–6.77)	6.04 (4.58–7.51)	0.37 (−0.55–1.29)	0.43
C-peptide (ng/ml)	29	3.79 (3.08–4.50)	3.85 (3.03–4.67)	0.07 (−0.30–0.43)	0.72
TC (mmol/L)	34	5.83 (5.48–6.17)	5.73 (5.32–6.14)	−0.08 (−0.33–0.16)	0.51
HDL (mmol/L)	34	1.49 (1.32–1.66)	1.49 (1.31–1.66)	−0.01 (−0.06–0.04)	0.78
LDL (mmol/L)	34	4.03 (3.68–4.38)	3.88 (3.55–4.21)	−0.03 (−0.21–0.16)	0.78
TG (mmol/L)	34	1.62 (1.25–1.99)	1.63 (1.24–2.02)	0.03 (−0.13–0.19)	0.74

Abbreviations: BMI, body mass index; BMR, basal metabolic rate; CI, confidence interval; Predicted BMR calculated with Harris-Benedict equation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MD, mean difference between chilli and bland diet while adjusting for order and period of the diet; *n*, number of subjects; RQ, respiratory quotient; TC, total cholesterol; TG, triglyceride; data compared with general linear model while adjusting for order and period of the diet.

Table 3 Some indicators of vascular function, separated by gender, at the end of the bland and the chilli diet

Variable (units)	*Mean (95% CI)*	*MD (95% CI)*	P	*Mean (95% CI)*	*MD (95% CI)*	P
	Men (n = 14)			*Women (n = 20)*		
SBP (mmHg)						
Bland	122 (116.50–128.21)			113 (105.28 to 119.96)		
Chilli	124 (116.85–130.94)	1.54 (−4.04–7.11)	0.59	114 (106.82–120.84)	0.81 (−2.36–3.99)	0.62
DBP (mmHg)						
Bland	81 (77.15–84.42)			74 (71.03 to 77.83)		
Chilli	81 (75.64–85.14)	−0.39 (−3.51–2.73)	0.81	74 (70.61–77.01)	−0.79 (−2.56–0.98)	0.38
AG (mm Hg)						
Bland	7 (5.07–9.57)			9 (7.39 to 9.99)		
Chilli	9 (6.04–11.17)	1.29 (−0.61–3.18)	0.18	10 (7.64–12.27)	1.20 (−0.96–3.37)	0.28
SEVR (%)						
Bland	182 (163.76–199.38)			181 (163.44 to 198.76)		
Chilli	188 (170.89–205.89)	6.82 (−0.09–13.73)	0.05	177 (158.44–195.57)	−6.48 (−15.13–2.17)	0.14
HR (beats/min)						
Bland	63 (56.20–69.80)			61 (55.47 to 66.66)		
Chilli	61 (55.13–65.87)	−2.50 (−4.66 to −0.34)	0.02	61 (54.94–66.07)	0.08 (−2.00–2.16)	0.94
AIx@HR75 (%)						
Bland	17 (12.57–20.72)			23 (18.73 to 27.10)		
Chilli	17 (17.29–21.67)	0.64 (−2.94–4.23)	0.73	24 (18.22–29.06)	0.69 (−3.78–5.16)	0.76

Abbreviations: AG, augmentation; AIx@HR75, augmentation Index corrected for heart rate 75 beats/min; CI, confidence interval; DBP, diastolic blood pressure; HR, heart rate; MD, mean difference between chilli and bland adjusted for order and period effects; SEVR, subendocardial viability ratio; SBP, systolic blood pressure.

Sub-study
AIx@HR75 at baseline, 10 and 15 min after GTN intake was significantly lower ($P<0.05$) on the chilli diet than on the bland diet (Figure 2). However, there was no significant difference between the net-AUC responses to the two drugs (glyceryl trinitrate and salbutamol), between the two dietary periods.

Discussion

The purpose of the present study was to examine the effects of regular consumption of chilli on some indicators of metabolic and vascular function and CHD risk. The results show no significant effect of regular chilli consumption on metabolic rate or substrate oxidation, similar to two previous capsaicin studies (Lejeune *et al*., 2003; Belza and Jessen, 2005), although Belza and Jessen (2005) did report a 'trend' towards higher EE ($P=0.06$) after one week of regular capsaicin intake. This trend towards a higher EE observed in overweight and obese men may have been due to the synergistic effect of capsaicin with caffeine, tyrosine, calcium and green tea extract (Belza and Jessen, 2005).

The results of the present study are different from single meal (postprandial) studies, possibly due to the following reasons. The single meals trials, which reported higher EE after the meals containing chilli than the bland meals, were conducted using a small group of participants ($n=8$–13) with a mean BMI of 22–24 kg/m^2 (Henry and Emery 1986; Yoshioka *et al*., 1998; Yoshioka *et al*., 2001). However, the mean BMI of our subjects ($n=36$) was 26 kg/m^2 (range 18–35 kg/m^2). As the thermic responses to mixed meals may differ between lean and overweight/obese people (Segal *et al*., 1987; Matsumoto *et al*., 2001), the results from the single meal studies may not be relevant in the context of people with higher BMI who are trying to lose weight. In fact, Matsumoto *et al*. (2000) have shown energy expenditure to be significantly higher after a spicy meal (MD = 0.54 MJ; $P<0.01$) compared to the bland meal, in lean participants, whereas there was no significant difference (MD = 0.34 MJ) in obese participants, matched for fat mass with the lean.

It is also possible that with regular consumption of chilli, the higher EE is evident for only a short period of time but not medium-long-term – adaptability effect. Animal studies have shown that capsaicin pre-treatment inhibits capsaicin-induced catecholamine secretion (Watanabe *et al*., 1988). Conversely, a recent study in overweight and obese humans has shown no adaptation effect on EE (Belza *et al*., 2006). This study observed a significantly higher EE after a combined dose of bioactive compounds including caffeine, capsaicin, green tea extracts and calcium compared to a placebo, irrespective of whether the measurements were made after the first dose or after the last dose with regular consumption for eight weeks. It may be possible that the effects of capsaicin and other compounds in increasing the EE are produced acutely (just after the intake) but are not carried over to the post-absorptive/fasting states even with the regular consumption for 4–12 weeks, as observed in the present study and earlier reported by Lejeune *et al*. (2003).

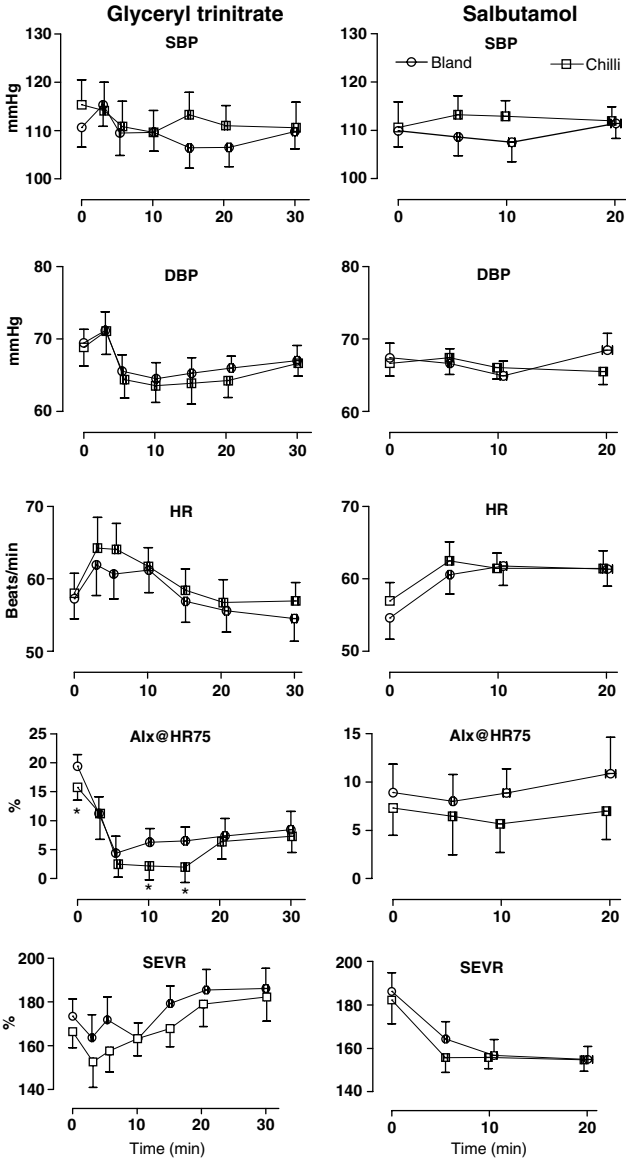

Figure 2 Effects of glyceryl-trinitrate and salbutamol on systolic blood pressure (SBP); diastolic blood pressure (DBP); heart rate (HR); augmentation index corrected for heart rate of 75 beats/min (AIx@HR75) and subendocardial viability ratio (SEVR) in week 3 of the chilli and the bland diet ($n=15$). Data as mean ± s.e.m.; *significantly different ($P<0.05$) between the chilli and the bland diet analysed using general linear model while adjusting for order and period of diet.

No significant change in serum lipids and lipoproteins, such as have been reported in some animal studies, were observed (Saito *et al*., 1999; Saito *et al*., 2000; Lee *et al*., 2003). This may be due to differences in species, or in total capsaicin intake. Effects in animal studies (guinea pig, turkey

and rat) were observed on feeding of a minimum of 3 mg/kg body weight of capsaicin (Negulesco *et al*., 1987; Negulesco *et al*., 1989; Lee *et al*., 2003), whereas the present study provided a mean of only 0.8 mg/kg body weight per day. Greater intake of chilli in humans may not be possible due to the overpowering taste and increased gastric motility. Although any side-effects (gastric motility) of regular consumption of 30 g of the chilli blend used in the present investigation disappeared after the first week of intake, participants reported that regular ingestion of higher amounts would not be possible over a long period.

It is also possible that the consumption of 30 g of the chilli blend in a single meal or distributed over two to three meals a day could give different results. However, the aim of the present investigation was to evaluate the effects of a certain amount of chilli blend intake per day. Allowance of choice regarding distribution of chilli throughout the day was to gain better compliance, as most participants ($\sim$90%) were naive or infrequent consumers of chilli.

Chilli induces vasodilatation, possibly through an increased release of calcitonin gene-related peptide and/ or increased synthesis of nitric oxide (Chen *et al*., 1996; Oroszi *et al*., 1999). The lower AIx (in the sub-study) at baseline and subsequent time points, but no significant difference in the net-AUC between the chilli and bland diet after the drugs suggests that chilli may produce vasodilatation at baseline with regular consumption for 2–3 weeks, but does not increase or hinder the acute effects of the two major vasodilatory drugs. This lowered AIx was not evident at the end of 4 weeks, which again raised the possibility of short-term effect. Cutaneous dilatation with transdermal use of capsaicin depends on age, gender and applied dose of capsaicin with young men being positive responders compared to older men; and women being non-responders (Munce and Kenney 2003; Ferrell *et al*., 2004) and no effect with repeated use (Roberts *et al*., 1992). Capsaicin significantly delays ST depression time during exercise test in male patients with stable coronary disease, indicating a possible role of capsaicin in arteriolar vasodilatation in men (Fragasso *et al*., 2004). Although there was no significant difference in the whole group, the resting heart rate was lower and SEVR (a measure of myocardial perfusion) was higher in men, after 4 weeks of regular chilli consumption. Elevated resting heart rate is a risk factor for cardiovascular disease, especially in white men (Kannel *et al*., 1987; Gillum *et al*., 1991). As coronary perfusion occurs during the diastolic phase, reducing heart rate is thought to have anti-ischemic effects (Danchin and Aly, 2004). Longer diastolic time also suggests a longer perfusion time (Fokkema *et al*., 2005). Diastolic time in men on the chilli diet was higher, but not statistically significant ($P=0.08$). These results suggest that chilli may play a role in improving coronary perfusion in men. However, research with a larger sample size is required to accept or reject this hypothesis.

In conclusion, the regular supplementation of chilli for 4 weeks (on a weight maintenance regime) has no obvious beneficial or harmful effects on the measured metabolic CVD risk factors. However, further research is warranted, particularly in men, to evaluate any the potentially beneficial effects of regular chilli consumption on vascular function.

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References

Belza A, Frandsen E, Kondrup J (2006). Body fat loss achieved by stimulation of thermogenesis by a combination of bioactive food ingredients: a placebo-controlled, double-blind 8-week intervention in obese subjects. *Int J Obes (London)*.

Belza A, Jessen AB (2005). Bioactive food stimulants of sympathetic activity: effect on 24-h energy expenditure and fat oxidation. *Eur J Clin Nutr* **59**, 733–741.

Buckberg GD, Fixler DE, Archie JP, Hoffman JI (1972). Experimental subendocardial ischemia in dogs with normal coronary arteries. *Circ Res* **30**, 67–81.

Chen IJ, Yeh JL, Lo YC, Sheu SH, Lin YT (1996). Capsinolol: the first beta-adrenoceptor blocker with an associated calcitonin gene-related peptide releasing activity in the heart. *Br J Pharmacol* **119**, 7–14.

Danchin N, Aly S (2004). Heart rate reduction: a potential target for the treatment of myocardial ischaemia. *Therapie* **59**, 511–515.

Eckel RH, Grundy SM, Zimmet PZ (2005). The metabolic syndrome. *Lancet* **365**, 1415–1428.

Ferrell WR, Wong BB, Lockhart JC, Ramsay JE (2004). Gender differences in regional cutaneous microcirculatory responses to capsaicin. *Fundam Clin Pharmacol* **18**, 195–200.

Fokkema DS, VanTeeffelen JW, Dekker S, Vergroesen I, Reitsma JB, Spaan JA (2005). Diastolic time fraction as a determinant of subendocardial perfusion. *Am J Physiol Heart Circ Physiol* **288**, H2450–H2456.

Fragasso G, Palloshi A, Piatti PM, Monti L, Rossetti E, Setola E *et al*. (2004). Nitric-oxide mediated effects of transdermal capsaicin patches on the ischemic threshold in patients with stable coronary disease. *J Cardiovasc Pharmacol* **44**, 340–347.

Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18**, 499–502.

Gillum RF, Makuc DM, Feldman JJ (1991). Pulse rate, coronary heart disease, and death: the NHANES I Epidemiologic Follow-up Study. *Am Heart J* **121**, 172–177.

Henry CJ, Emery B (1986). Effect of spiced food on metabolic rate. *Hum Nutr Clin Nutr* **40**, 165–168.

Kannel WB, Kannel C, Paffenbarger Jr RS, Cupples LA (1987). Heart rate and cardiovascular mortality: the Framingham Study. *Am Heart J* **113**, 1489–1494.

Kawada T, Hagihara K, Iwai K (1986a). Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J Nutr* **116**, 1272–1278.

Kawada T, Watanabe T, Takaishi T, Tanaka T, Iwai K (1986b). Capsaicin-induced beta-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the

respiratory quotient, and substrate utilization. *Proc Soc Exp Biol Med* **183**, 250–256.
Lee CY, Kim M, Yoon SW, Lee CH (2003). Short-term control of capsaicin on blood and oxidative stress of rats *in vivo*. *Phytother Res* **17**, 454–458.
Lejeune MP, Kovacs EM, Westerterp-Plantenga MS (2003). Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *Br J Nutr* **90**, 651–659.
Lim K, Yoshioka M, Kikuzato S, Kiyonaga A, Tanaka H, Shindo M *et al.* (1997). Dietary red pepper ingestion increases carbohydrate oxidation at rest and during exercise in runners. *Med Sci Sports Exerc* **29**, 355–361.
Matsumoto T, Miyawaki C, Ue H, Kanda T, Yoshitake Y, Moritani T (2001). Comparison of thermogenic sympathetic response to food intake between obese and non-obese young women. *Obes Res* **9**, 78–85.
Matsumoto T, Miyawaki C, Ue H, Yuasa T, Miyatsuji A, Moritani T (2000). Effects of capsaicin-containing yellow curry sauce on sympathetic nervous system activity and diet-induced thermogenesis in lean and obese young women. *J Nutr Sci Vitaminol (Tokyo)* **46**, 309–315.
Moller DE, Kaufman KD (2005). Metabolic syndrome: a clinical and molecular perspective. *Annu Rev Med* **56**, 45–62.
Munce TA, Kenney WL (2003). Age-specific skin blood flow responses to acute capsaicin. *J Gerontol A Biol Sci Med Sci* **58**, 304–310.
Negulesco JA, Lohse CL, Hrabovsky EE, Boggs MT, Davis DH (1989). Dihydrocapsaicin (DC) protects against serum hyperlipidemia in guinea pigs fed a cholesterol-enriched diet. *Artery* **16**, 174–188.
Negulesco JA, Noel SA, Newman HA, Naber EC, Bhat HB, Witiak DT (1987). Effects of pure capsaicinoids (capsaicin and dihydrocapsaicin) on plasma lipid and lipoprotein concentrations of turkey poults. *Atherosclerosis* **64**, 85–90.
Oroszi G, Szilvassy Z, Nemeth J, Tosaki A, Szolcsanyi J (1999). Interplay between nitric oxide and CGRP by capsaicin in isolated guinea-pig heart. *Pharmacological Research* **40**, 125–128.
O'Rourke MF, Gallagher DE (1996). Pulse wave analysis. *J Hypertens Suppl* **14**, S147–S157.
Roberts RG, Westerman RA, Widdop RE, Kotzmann RR, Payne R (1992). Effects of capsaicin on cutaneous vasodilator responses in humans. *Agents Act* **37**, 53–59.
Saito A, Nakamura K, Hori Y, Yamamoto M (1999). Effects of capsaicin on serum triglycerides and free fatty acid in olive oil treated rats. *Int J Vitam Nutr Res* **69**, 337–340.
Saito A, Nakamura K, Hori Y, Yamamoto M (2000). Effects of capsaicin on biliary free fatty acids in rats. *Int J Vitam Nutr Res* **70**, 19–23.
Schaefer EJ, Lichtenstein AH, Lamon-Fava S, Contois JH, Li Z, Rasmussen H *et al.* (1995a). Efficacy of a National Cholesterol Education Program Step 2 Diet in Normolipidemic and Hypercholesterolemic Middle-Aged and Elderly Men and Women. *Arterioscler Thromb Vasc Biol* **15**, 1079–1085.
Schaefer EJ, Lichtenstein AH, Lamon-Fava S, McNamara JR, Schaefer MM, Rasmussen H *et al.* (1995b). Body weight and low-density lipoprotein cholesterol changes after consumption of a low-fat ad libitum diet. *JAMA* **274**, 1450–1455.
Segal KR, Gutin B, Albu J, Pi-Sunyer FX (1987). Thermic effects of food and exercise in lean and obese men of similar lean body mass. *Am J Physiol* **252**, E110–E117.
Srinivasan MR, Chandrasekhara N (1992). Comparative influence of vanillin & capsaicin on liver & blood lipids in the rat. *Indian J Med Res* **96**, 133–135.
Sundell J (2005). Obesity and diabetes as risk factors for coronary artery disease: from the epidemiological aspect to the initial vascular mechanisms. *Diabetes Obes Metab* **7**, 9–20.
Suzuki T, Wada S, Tomizawa N, Kamata R, Saito S, Sato I *et al.* (1998). A possible role of nitric oxide formation in the vasodilatation of rabbit ear artery induced by a topically applied Capsaicin analogue. *J Vet Med Sci* **60**, 691–697.
Tani Y, Fujioka T, Sumioka M, Furuichi Y, Hamada H, Watanabe T (2004). Effects of capsinoid on serum and liver lipids in hyperlipidemic rats. *J Nutr Sci Vitaminol (Tokyo)* **50**, 351–355.
Watanabe T, Kawada T, Iwai K (1988). Effect of capsaicin pretreatment on capsaicin-induced catecholamine secretion from the adrenal medulla in rats. *Proc Soc Exp Biol Med* **187**, 370–374.
Watanabe T, Kawada T, Yamamoto M, Iwai K (1987). Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. *Biochem Biophys Res Commun* **142**, 259–264.
Weir JB (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* **109**, 1–9.
Westerbacka J, Tamminen M, Cockcroft J, Yki-Jarvinen H (2004). Comparison of *in vivo* effects of nitroglycerin and insulin on the aortic pressure waveform. *Eur J Clin Invest* **34**, 1–8.
Wilkinson IB, Cockcroft JR, Webb DJ (1998). Pulse wave analysis and arterial stiffness. *J Cardiovasc Pharmacol* **32**, S33–S37.
Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ (2000). The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol* **525**, 263–270.
Yoshioka M, Doucet E, Drapeau V, Dionne I, Tremblay A (2001). Combined effects of red pepper and caffeine consumption on 24 h energy balance in subjects given free access to foods. *Br J Nutr* **85**, 203–211.
Yoshioka M, Imanaga M, Ueyama H, Yamane M, Kubo Y, Boivin A *et al.* (2004). Maximum tolerable dose of red pepper decreases fat intake independently of spicy sensation in the mouth. *Br J Nutr* **91**, 991–995.
Yoshioka M, Lim K, Kikuzato S, Kiyonaga A, Tanaka H, Shindo M *et al.* (1995). Effects of red-pepper diet on the energy metabolism in men. *J Nutr Sci Vitaminol (Tokyo)* **41**, 647–656.
Yoshioka M, St-Pierre S, Drapeau V, Dionne I, Doucet E, Suzuki M *et al.* (1999). Effects of red pepper on appetite and energy intake. *Br J Nutr* **82**, 115–123.
Yoshioka M, St-Pierre S, Suzuki M, Tremblay A (1998). Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. *Br J Nutr* **80**, 503–510.