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LACK OF EFFECT OF COLD WATER PRAWNS ON PLASMA CHOLESTEROL AND LIPOPROTEINS IN NORMO-LIPIDAEMIC MEN

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Abstract – Objective: Dietary guidelines for the prevention of coronary heart disease (CHD) have restricted the intake of foods rich in dietary cholesterol, on the grounds that the dietary cholesterol will increase blood cholesterol. In the case of shellfish, this recommendation may limit the intake of a valuable dietary source of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA). The objective of this study was to undertake a dietary intervention to determine the effects of cold water prawns on plasma lipids and lipoproteins. Methods: 23 healthy male subjects were randomised to receive either 225 g of cold water prawns or an equivalent weight of fish ('crab') sticks as a control for 12 weeks in a cross-over design. Blood samples were taken at the beginning and end of each intervention for the determination of plasma lipids and lipoproteins by routine enzymatic assays and iodixanol density gradient centrifugation respectively. Results: The diets were well matched for the intake of total energy and macronutrients, and body weight remained stable throughout the study. The prawn intervention increased the intake of dietary cholesterol to 750 mg/d against 200mg/d on the control. The intake of LC n-3 PUFA from prawns was estimated to be between 0.5-0.7g/d. The consumption of prawns produced no significant effects on the concentration of plasma total or LDL cholesterol, triacylglycerol, HDL cholesterol or apolipoproteins A-I and B relative to the control, or within each intervention group over time. There was also no significant effect on LDL density (particle size) relative to the control, or any difference between and within treatments in total plasma lipoprotein profiles by density gradient centrifugation. Conclusion: These findings provide evidence to suggest that the consumption of cold water prawns, at least in healthy, male subjects, should not be restricted on the grounds of this seafood producing an adverse effect on plasma LDL cholesterol.

Key words: Coronary heart disease, dietary cholesterol, n-3 polyunsaturated fatty acids, low density lipoprotein cholesterol, cold water prawns.

INTRODUCTION

Dietary guidelines for the prevention of CHD have traditionally restricted the intake of foods rich in dietary cholesterol, on the grounds that the dietary cholesterol will increase blood cholesterol and thus CHD risk. The scientific basis for this argument has been challenged recently because of a lack of evidence to link dietary cholesterol, chiefly from eggs, with blood cholesterol and CHD risk (11,9). The impact of dietary cholesterol from eggs on plasma LDL cholesterol has been shown to be negligible in terms of CHD risk, and also relatively minor in comparison to the cholesterol-raising properties of saturated fatty acids (12,7,14,13). However, there is less evidence for other dietary sources of cholesterol, including certain shellfish. The controversy surrounding dietary cholesterol has negatively influenced the consumption of shellfish, and continues to threaten the future intake of LC n-3 PUFA from this rich, and potentially more sustainable, source of these fatty acids than oily fish (**Table 1**). The existing evidence for the effects of shellfish on blood lipids from dietary intervention trials in humans is limited (3,2,1,6). Studies to date have focused on the impact of shellfish, including prawns (shrimp) on serum cholesterol and lipoproteins

Abbreviations: **ANOVA,** analysis of variance; **CHD,** coronary heart disease; **CIU,** clinical investigation unit; **DHA,** docosahexaenoic acid; **DBP,** Diastolic blood pressure; **EPA,** eicosapentaenoic acid; **HDL,** high density lipoprotein; **LC n-3 PUFA,** long chain n-3 polyunsaturated acids; **lbLDL,** large, buoyant LDL; **LDL,** low density lipoprotein cholesterol; **sdLDL,** small, dense LDL; **SBP,** Systolic blood pressure; **SD,** standard deviation; **TAG,** triacylglycerol; **TC,** total cholesterol; **VLDL,** very low density lipoprotein.

but these studies have been few in number, examined small numbers of subjects, and often included run-in periods on low cholesterol / low fat diets that influenced plasma lipids before the main dietary intervention, and were thus unrepresentative of the habitual diet. The present study was designed to determine the effects of cold water prawns on plasma lipids and lipoproteins of healthy male subjects against a control, and a background of their habitual diet.

MATERIAL AND METHODS

Subjects

The power and sample size for the study was based on a reported change in plasma LDL cholesterol of 0.16 mmol/l (5% reduction, SD=0.2 mmol/l) in 18 normo-lipidaemic men fed 225g/d of shrimp for 21 days (6). This indicated that a sample size of 19 subjects, at 90% power, would be required to detect a significant difference at the 5% level ($p<0.05$). The aim was therefore to recruit a total of 30 subjects to allow for a subject drop-rate of up to 30%. A total of 25 healthy male subjects (mean age 41 years (range 19-67), mean body weight 82 ± 12 kg (SD), mean BMI 25.8 ± 3.1kg/m^2), were recruited from members of staff and postgraduate students at the University of Surrey. All subjects were normo-lipidaemic (total cholesterol <6.5mM, triacylglycerol <2.3mM), normo-tensive (mean SBP/DBP 125/73 mmHg), and free of dietary supplements (e.g. fish-oil), or medication or any medical condition known to affect lipid metabolism or impair their ability to undertake the dietary intervention, such as shellfish allergy or a dislike of prawns or fish. Subjects were also excluded if they were actively trying to lose weight or had lost more than 3kg in the preceding 3 months.

Study design

The study had a randomly controlled, cross-over design in which subjects were instructed to incorporate either cold water prawns (225g) or an equivalent weight of fish ('crab') sticks as a control, into their habitual diet for 4 weeks. At the end of this first intervention, the subjects entered a wash-out period of 4 weeks during which they continued to consume their habitual diet, without any source of fish or seafood. Subjects then crossed-over to the alternative treatment in a second intervention for a further 4 weeks (**Figure 1**). Subjects were instructed to maintain their normal, habitual diet throughout all three phases of the study, and not to consume any other sources of fish or shellfish other than that supplied. They were also given strict instructions to maintain all other lifestyle factors throughout the 12 weeks of study, including physical activity.

Study visits

Subjects attended an initial pre-study screening visit, after an overnight fast (12h), at the Clinical Investigation Unit (CIU) at the University of Surrey. During this first visit, subjects completed a diet and health questionnaire, provided anthropometric and blood pressure measurements and a blood sample, and were given instruction on how to complete a 3-day diet diary. Subjects who met the entry criteria were invited to participate in the study, and attend the CIU, after an overnight fast, on four further occasions; at the beginning and end of each 4 week intervention period. At the second pre-study visit, the study was explained in full and subjects were asked to read the Volunteer Information Sheet and to provide written consent to participate. Subjects were then randomised, using a random number generator, to one of the two interventions. At each visit, anthropometric measures were repeated and a 30ml blood sample was taken. Subjects were also asked to complete two further 3-day diet diaries towards the end of each intervention period, and to include at least one weekend day in each 3-day period.

Assessment of dietary intakes and laboratory analyses

The dietary intake of macronutrients was determined from 3-day diet diaries (D_1-D_3, **Figure 1),** using 'WinDiet' software (Robert Gordon University, UK, 2005). At each study visit, blood was taken from an ante-cubital vein and collected into EDTA. All plasma samples were separated and stored in 1ml aliquots at -80^{O}C before laboratory analysis which was undertaken at the end of the first intervention, and repeated again on all samples at the end of the study (12 weeks). Plasma lipids; total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C) and small, dense LDL (sdLDL), were measured by commercially available enzymatic assays, and apoplipoproteins A-I and B by immunoturbidimetric assays (Randox, UK). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (8). All measurements were performed at the University of Surrey on an ILab 650 auto-analyser (Instrumentation Laboratory, UK). Plasma lipoproteins were also separated by iodixanol density gradient centrifugation and fractionated to produce a total lipoprotein profile (VLDL, total LDL, small, dense and large buoyant LDL (sd, lbLDL) and HDL on the basis of the cholesterol concentration of individual fractions as previously described (5). This analysis was performed at Liverpool John Moores University. Inter and intra-coefficients of variation for lipid and apoprotein assays were all <2%. These values for the lipoproteins separated by iodixanol centrifugation were <3% and <1% respectively (5).

Statistical analysis

The distribution of all data was tested for normality by the use of normal probability plots, and when shown to be asymmetric by the Anderson-Darling test, logarithmically transformed ($\log_e$) to permit parametric testing (TG, HDL-C, apo A-I). Data that could not be normalised (LDL-C, sdLDL) was analysed non-parametrically. Data is expressed as arithmetic means, standard deviations (SD) and medians. Differences between the prawn and control groups, at baseline and after 4 weeks of intervention (T_0 versus T_4), were examined by paired student *t*-test for dependent samples, and by Wilcoxon ranked sign test for non-normally distributed data. Difference in total lipoprotein profiles were examined by a two-way ANOVA using treatment (prawn versus control) and lipoprotein fractions as factors, and by General Linear Model, using treatment as the factor and lipoprotein fractions as the repeated measure. The results from each intervention period were also examined separately to check for order effects of the two treatments. Statistical analysis was performed using Minitab® software (version 14). A probability level of $p<0.05$ was considered significant.

RESULTS

A total of 23 subjects completed both

Table 1. Amounts of long chain n-3 polyunsaturated in selected shellfish (milligrams of EPA + DHA/100g meat)

	Autumn	Spring
Crustaceans		
Cold water prawns hand peeled	547	658
processed as eaten	267	332
Warm water prawns (*P vannemei*)	181	197
Lobster	415	413
Crab (*Cancer pagurus*) brown meat	2,450	2,474
Crab (*Cancer pagurus*) white meat	174	307
Molluscs		
Mussels (*Mytilus edulis*)	992	372
Pacific oysters (*Crassostrea gigas*)	1,739	1,463
Cockles (*Cerastoderma edule*)	426	258
Squid	537	878
Reference values for fish		
Cod	170-260	
Herring	1,600-2,350	
Mackerel	1,300-2,600	

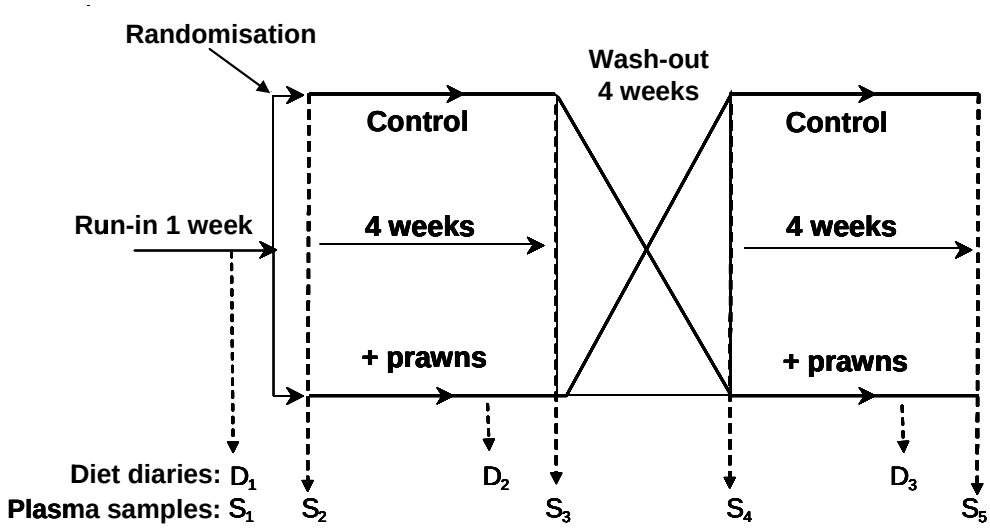

Figure 1. Study design with diet diaries and blood sampling protocol

dietary intervention periods. Dietary intakes showed that the subjects were compliant with the study protocol, and successful in incorporating the prawns and fish sticks into their habitual diet. The daily intake of dietary cholesterol from 225g of machine processed cold water prawns was calculated to be 295mg, and the intake of LC n-3 PUFA between 0.6-0.7 g (EPA+DHA). In contrast, the fish sticks (control), which were composed of 'surimi' (white-fleshed fish pulverised to a paste to mimic shellfish meat), delivered only traces of cholesterol and LC n-3 PUFA. The fish sticks had a higher content of energy and fat (115 Kj, 1.6g fat/100g versus 62 Kj/100g, 0.74g fat/100g), but a lower content of protein than the prawns (7.8 g/100g versus 13.9 g/100g). There was no significant difference in the daily intake of total energy or macronutrients between the prawn and control interventions but a significantly higher intake of dietary cholesterol on prawns versus the control ($p<0.001$, **Table 2**).

Body weight, BMI, and systolic and diastolic blood pressures were well matched at baseline and unaffected by the dietary interventions (data not shown). There was no significant difference in the concentration of plasma TC, TAG, HDL-C, LDL-C, sdLDL or apoproteins A-I and B between the prawn and control groups at baseline, before the intervention, or after 4 weeks of dietary intervention **(Table 3).** There was no difference in the ratio of TC: HLD-C, or any of these variables over time (within each intervention), and no order effects of the treatments. Total plasma lipoprotein profiles, as measured by density gradient centrifugation, revealed significant variation between individual subjects ($p<0.001$) but no overall effect of the dietary interventions (prawns versus control) on the distribution of lipoprotein density, and more specifically, large, buoyant and small, dense LDL **(Figure 2)**.

Table 2. Daily intake of macronutrients, fats and alcohol during the prawn and control interventions

	Prawn		Control	
	Mean	SD	Mean	SD
Energy (Mj)	8.16	1.6	8.8	2.7
Carbohydrate (% energy)	43	7	49	10
Protein (% energy)	24	4	19	5
Fat (% energy)	30	5	29	8
SFA (g)	19	5	21	10
PUFA (g)	14	8	11	7
MUFA (g)	22	6	22	10
Cholesterol (Mg)	794	186	275	92
Alcohol (% energy)	7	8	7	6

Dietary intake data was based on the analysis of 3-day food diaries (D_2 and D_3 in Figure 1). The composition of the cold water prawns and fish sticks (control) was provided by the manufacturer of each product.

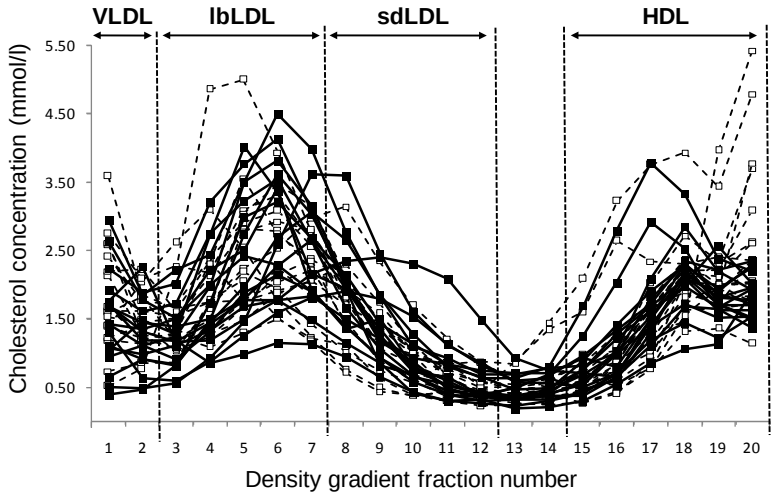

Figure 2. Total plasma lipoproteins and light and dense LDL as separated by iodixanol density gradient centrifugation (5). Profiles represent post-prawn (unbroken line with filled symbols) and post-control (broken line with open symbols) interventions. Profiles consist of VLDL, large, buoyant and small, dense LDL, and HDL.

Table 3. Concentration of plasma lipids, lipoproteins and apolipoproteins before and after 4 weeks of dietary intervention with cold water prawns and control

	Prawn				Control			
	T_0		$T_{4\ weeks}$		T_0		$T_{4\ weeks}$	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total cholesterol (mmol/L)	5.08 (4.80)	1.08	5.11 (5.15)	0.83	5.12 (5.05)	0.92	5.09 (5.05)	0.81
LDL-cholesterol (mmol/L)[2]	2.94 (2.91)	0.87	3.02 (3.12)	0.65	2.98 (3.10)	0.69	2.98 (3.17)	0.64
HDL-cholesterol (mmol/L)[1]	1.56 (1.43)	0.44	1.57 (1.51)	0.39	1.58 (1.47)	0.44	1.55 (1.45)	0.42
Triacyglycerol (mmol//L)[1]	1.26 (1.16)	0.48	1.16 (1.07)	0.43	1.22 (1.11)	0.57	1.23 (1.17)	0.42
sdLDL-cholesterol (mmol/L)[2]	0.41 (0.34)	0.31	0.41 (0.43)	0.22	0.39 (0.38)	0.17	0.57 (0.43)	0.43
Apoprotein A-I (g/l)[1]	1.41 (1.37)	0.23	1.36 (1.33)	0.31	1.38 (1.33)	0.20	1.38 (1.41)	0.20
Apoprotein B (g/l)	0.98 (0.96)	0.22	0.90 (0.90)	0.16	0.92 (0.94)	0.18	0.95 (0.96)	0.16

Values in parentheses represent medians. Differences between treatments ([1]Log_e transformed means for Prawns versus Control) were tested at baseline (T_0) and after 4 weeks of intervention (T_{4weeks}) with a one-sample students *t*-test . [2]Differences between treatments (medians for Prawns versus Control) were tested using a one-sample Wilcoxon signed rank test.

DISCUSSION

In 1963, the serum cholesterol of rabbits was shown to increase dramatically when they were fed a mixture of chow and dried shrimp, an effect that promoted severe atherosclerosis in a matter of weeks (4). At the time, this finding fuelled the historic link between dietary cholesterol and coronary atherosclerosis, but in retrospect only serves to confirm the extreme sensitivity of this animal species to dietary cholesterol. The same author went on to examine the effects of a range of shellfish on serum cholesterol in humans adapted to a low cholesterol diet but showed only a mild increase in LDL cholesterol in normo-cholesterolaemic participants. This mild effect was reported to be less than other cholesterol-containing foods (3). In the present study, a diet that included 225g of cold water prawns, delivering approximately 0.8g (range 0.4-1.1g) of dietary cholesterol per day for 4 weeks, produced no significant effect on either plasma total or LDL cholesterol relative to a diet containing an equivalent weight of fish sticks, in healthy, male subjects. Fish or 'crab' sticks were considered to be an appropriate control for prawns, primarily because they contain only traces of cholesterol and LC n-3 PUFA, but also because of their similarity to prawns in the way that they could be incorporated into the habitual diets of the subjects. There was no evidence from dietary intakes, or change in body weight, to suggest that the prawns influenced the intake of energy, or any other macronutrients as compared to the control. However, both diets were unusually low in energy and fat, and high in protein relative to the UK population (15). This could reflect under reporting, but also an influence of both interventions on habitual dietary intake, though body weight was maintained throughout the study. One explanation for the apparent lack of effect of dietary cholesterol on LDL cholesterol might be through an inhibition of cholesterol absorption produced by non-cholesterol sterols in the shellfish (2). However, unlike certain other shellfish such as oysters, prawns contain a relatively small amount of non-cholesterol sterols, making an effect on cholesterol absorption unlikely in this case. Mixtures of shellfish have also been shown to produce either no effect on blood lipids (shrimp and squid) or lower the ratio of LDL to HDL (oysters and

mussels) (1). The specific effect of 300g prawns (590mg dietary cholesterol/day), on blood cholesterol has been compared with an equivalent amount of dietary cholesterol from two eggs a day. In this earlier study, prawns increased plasma LDL but to a lesser extent than the eggs (7% versus 10%), and increased HDL (12%). From the resultant decrease in the ratio of LDL to HDL it was concluded that prawns were a suitable food to be included in 'heart healthy' guidelines (6). In the present study, the prawns were estimated to have delivered between 0.6-0.7g of LC n-3 PUFA per day. However, there was no evidence of any changes in plasma lipids that are characteristic of these dietary fatty acids; including a reduction in plasma TAG, as previously reported in the comparison between prawns and eggs (6), or an increase in LDL particle size (10). This finding is perhaps predictable in view of the healthy, normo-lipidaemic status of the subjects at the beginning of the trial.

In conclusion, this study provides evidence to show that cold water prawns produce no significant effect on plasma LDL cholesterol, and thus should not be excluded from the diet of healthy, normo-lipidaemic men on the grounds of a reputed, cholesterol-raising effect.

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Other articles in this theme issue include references (16-27).

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