Red algal cellular biomass lowers circulating cholesterol concentrations in Syrian golden hamsters consuming hypercholesterolaemic diets

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Preliminary evidence suggests that consumption of *Porphyridium cruentum* (PC) biomass results in hypocholesterolaemic effects; however, mechanisms responsible have not been elucidated. The aim of the present study was to determine whether PC biomass lowers circulating cholesterol concentrations, dose dependently, in hamsters fed hypercholesterolaemic diets for 28 d and determine whether cholesterol biosynthesis is affected. Biomass added to diets at 2·5, 5 and 10 % resulted in 14, 38 and 53 % reductions ($P < 0.001$) in total plasma cholesterol, respectively, compared with a control diet. Similarly, non-HDL-cholesterol concentrations in the 5 and 10 % PC groups were reduced ($P < 0.001$) 28 and 45 %, respectively, *v.* controls. These effects were unrelated to cholesterol fractional synthesis rate (FSR), as this did not differ between either treatment or control animals. PC consumption had no effect on food intake, plasma glucose concentrations or energy expenditure, but percentage of body fat was lower ($P < 0.001$) in the 5 and 10 % PC groups compared with controls. These data show that PC reduces total plasma cholesterol and non-HDL-cholesterol when incorporated into the diet at levels as low as 2·5 %. The mechanism of action for this reduction may be related to increased excretion since food intakes and cholesterol FSR were not reduced in the animals receiving the PC. In conclusion, the use of PC biomass reduces circulating cholesterol, dose dependently, in hypercholesterolaemic hamsters but not via reductions in cholesterol FSR. There is potential for the use of this biomass as a functional ingredient to aid in the management of blood cholesterol concentrations.

Cholesterol lowering: Lipoproteins: Cholesterol biosynthesis: Animal models

Hypercholesterolaemia, specifically elevated LDL-cholesterol concentrations, represent a potent risk factor for the development of CVD[1]. Increasingly, individuals are integrating specific cholesterol-lowering foods and supplements into a routine of diet modification and exercise as primary prevention of hypercholesterolaemia[2]. The unique nutritive composition of the red algae *Porphyridium cruentum* (PC), including water-soluble sulphated polysaccharides and long-chain PUFA, has been proposed to possess lipid-lowering activity[3].

Dietary consumption of red algae is common in Asian countries, while North American and European food industries utilise polysaccharide components of red algae from the Rhodophyta family, to which PC belongs, as stabilisers, fillers or colourants[4]. The PC biomass has already been shown to exhibit antioxidant properties within *in vitro* cell culture models[5]. These properties appear to be related to the combination of glycoprotein and sulphated carbohydrate content as well as their unique polymeric structure. Sulphated carbohydrates have also been shown in increase colonic mucous secretion in rats[6], a physiological function that may increase faecal output and decrease intestinal transit time. Furthermore, a number of micro-organisms in the human colon are known to

ferment indigestible polysaccharides of algal origin[7]. The by-products of this fermentation can impact the colonic micro-environment, changing in redox state and pH[7]. Additionally, PC total fatty acid content has a high proportion of the long-chain PUFA, arachidonic acid (20 : 4n-6) and EPA (20 : 5n-3)[3,8,9], both known to affect circulating TAG concentrations, albeit in opposite fashions[10,11]. Accordingly, we expect that changes in circulating TAG could result when PC is used as a dietary supplement on regular basis.

In a feeding study in chickens exploring the potential of PC biomass to reduce egg yolk cholesterol concentrations, the authors observed a decrease in circulating cholesterol concentrations[12]. Similarly, a feeding study conducted in rats showed a link between consumption of PC and decreased circulating cholesterol concentrations[13]. Isolated sulphated polysaccharide fraction from the PC had the most pronounced effect on plasma cholesterol concentrations and neutral sterols excreted in faeces[13]. However, consumption of the whole-cell biomass from PC *v.* the isolated polysaccharides resulted in the largest effect on excretion of bile salts[13]. These data indicate a discrepancy, as to what aspect of cholesterol trafficking PC consumption actually targets, given that

the various fractions of the biomass appear to behave differently. Therefore, establishing how PC affects cholesterol biosynthesis becomes important in determining the mechanism behind the hypocholesterolaemic effect. Moreover, cholesterol management in hamsters is more similar to the human situation as both human subjects and hamsters carry excess cholesterol in LDL particles, unlike other rodents that carry very high concentrations of cholesterol in HDL particles.

While the data presented by Dvir *et al.*[13] suggest increased cholesterol and bile salt excretion as the major consequence of consuming the algae, the actual mechanism behind the cholesterol reductions has yet to be explored. Bioactive components of this algal biomass are likely to be absorbed and could disrupt normal cholesterol biosynthesis. Therefore, the purpose of the present study was to determine whether the cholesterol-lowering effect of PC consumption is dose dependent and how cholesterol synthesis rate is affected by the algal consumption in a diet-induced hypercholesterolaemic hamster model.

Methods and materials

Study design and animals

Sixty golden Syrian hamsters (*Mesocricetus auratus*; *n* 15) were randomised to receive either control hypercholesterolaemia-inducing diet alone, formulated in our laboratory (Table 1), or the same diet with added PC biomass. The PC was prepared for incorporation into the diet Solazyme, Inc. (San Francisco, CA, USA) by first disrupting cell integrity by microfluidisation followed by lyophilisation. The PC was then added to the diet at an increasing percentage (w/w; 2·5, 5 and 10 %) at the expense of maize starch. Table 2 illustrates the approximate increased daily intake of selected nutrients resulting from the added PC in the base diet.

All animals were then fed *ad libitum* a standardised hypercholesterolaemic diet[14,15] for 28 d with body weight and food consumption measured every 3 d. On day 25, energy expenditure, expressed as oxygen consumption per gram body weight, was measured by indirect calorimetry using a respiratory gas exchange system for rodents (MM-100 CWE, Inc., Pennsylvania, PA, USA). On day 28,

each hamster received an intraperitoneal injection of deuterated water (0·5 ml ^2H_2O, 99·9 atom percent excess: CDN Isotopes, Pointe-Claire, Que., Canada) to assess fractional cholesterol synthesis[16]. Exactly 2 h after injection, animals were anaesthetised with inhaled isoflurane and blood sampled by cardiac puncture. Animals were then euthanised with an overdose of sodium pentobarbital; body composition was determined immediately by dual emission X-ray absorptiometry. Liver, red blood cell (RBC) and faecal cholesterol were assayed in the 2·5 % PC group and their controls, but not in the higher intakes of PC. The study protocol was approved by the University of Manitoba Animal Care Committee in accordance to the Canadian Council on Animal Care Guidelines.

Blood chemistry

Blood was collected in heparinised tubes and separated into plasma and packed RBC by centrifugation at 1500 *g*. Plasma glucose, TAG, total cholesterol and HDL-cholesterol were measured using the Vitros Chemistry System 350 (Ortho-Clinical Diagnostics, Johnson & Johnson; New Orleans, LA, USA). Non-HDL-cholesterol is the difference between the measured total cholesterol and HDL-cholesterol and would include the sum of VLDL-, IDL- and LDL-cholesterol in the blood.

Erythrocyte, liver and faecal cholesterol concentrations

Approximately 0·5 g of liver, RBC and dried faeces were saponified with freshly prepared KOH–methanol at 100°C for 1 h. The non-saponifiable sterol fraction was extracted with petroleum diethyl ether and dried under N_2 gas. Before analysis, internal standard α-cholestane was added to each sample. The sterol fractions of liver and RBC were analysed for cholesterol concentrations using an Agilent 6890N GC fitted with an Agilent 5975 GC/MS captured total ion current monitor. Sterol fractions of the faecal samples were dissolved in chloroform and analysed using an Agilent 6890N gas chromatograph fitted with a flame ionisation detector[17]. A SAC-5 capillary column (30 m × 0·25 mm × 0·25 μm, Supelco, Bellefonte, CA, USA) was used for all of the sterol analyses.

Table 1. Hypercholesterolaemia-inducing diet composition

Ingredients	Control	2·5 % PC	5 % PC	10 % PC
	Total amount (g/kg)			
Casein	200	200	200	200
Maize starch	260	235	210	160
Algal biomass	0	25	50	100
Sucrose	330	330	330	330
Lard/sunflower mix	50	50	50	50
Cholesterol	2·5	2·5	2·5	2·5
Cellulose	100	100	100	100
DL-Methionine	5	5	5	5
Mineral mixture*	40	40	40	40
Vitamin mixture*	10	10	10	10
Choline bitartrate	2	2	2	2
BHT	0·2	0·2	0·2	0·2

PC, *Porphyridium cruentum* biomass; BHT, butylated hydroxytoluene.
* Vitamin mix AIN-76A (Harlan Teklad, Madison, WI, USA) and mineral mix AIN-93M (modified for hamsters, Harlan Teklad).

Table 2. Nutrient composition of *Porphyridium cruentum* (PC) and approximate daily intake for specific nutrients in test diets from algal biomass (intake above control diets) based on mean daily food intake

Nutrient	Algal biomass (g/100 g)	2·5 % PC	5 % PC	10 % PC
		Test diets (mg/d)		
Total carbohydrate	32·1	68·2	144·5	266·4
Crude fibre	0·39	0·83	1·8	3·2
Crude protein	34·1	72·5	153·5	283·0
Total lipid	6·53	13·9	29·4	54·2
16 : 0	1·58	3·4	7·1	13·1
20 : 4n-6	1·29	2·7	5·8	10·7
20 : 5n-3	1·27	2·7	5·7	10·5
Cholesterol	0·20	0·42	0·90	1·7

Intakes are based on mean food intakes for each treatment group and previously reported compositional data[3,24].

Fractional cholesterol synthesis

Approximately 0·5 g RBC were saponified with freshly prepared KOH–methanol at 100°C for 1 h and the sterol fraction was extracted with petroleum diethyl ether[16]. GC–thermal conversion–isotope ratio MS (Delta V Plus, Thermo Electron Corporation, Bremen, Germany) was then used to determine the $^2H/^1H$ ratio *v.* Vienna standard mean ocean water. The cholesterol precursor pool is taken as the mean plasma water ^2H enrichment, which was determined by thermal conversion elemental analyser-isotope ratio mass spectrometer from plasma prepared by membrane filtration and centrifugation removing proteins > 5 kDa. Cholesterol fractional synthesis rate (FSR) rates were calculated using the following equation[18]:

$$FSR\,(\%/d) = \frac{((\delta\,cholesterol/\delta\,plasma)\times 0.478)}{^2H\ incorporation\ period}\times 24\,h\times 100\,\%,$$

where δ is ^2H enrichment of cholesterol or plasma water above baseline and time refers to the 2 h ^2H incorporation period. The factor 0·478 is the fraction of hydrogen atoms per cholesterol molecule possibly labelled by a ^2H[18].

Statistical analysis

All outcomes were assessed by the generalised linear model univariate ANOVA using SPSS version 11 (SPSS, Inc., Chicago, IL, USA) including food intake as a covariate and study experiment as a fixed factor with $P<0.05$ considered significant. The variance was not homogeneous for cholesterol and non-HDL-cholesterol concentrations; therefore, these variables were log transformed for statistical testing but back transformed for the purpose of reporting in this paper. All data are reported as mean values with their standard errors.

Results

Weight gain and food intake

All hamsters were fed *ad libitum*, gaining weight similarly, with no difference observed in the rate of weight gain between either group over time or when expressed as percentage of weight gain from start (Table 3). Food intake did not differ from control for any treatment. However, the 5 % PC group mean daily food consumption (9·0 (SEM 0·2) g/d) was higher ($P<0.05$) than that of the 10 % PC group (8·3 (SEM 0·2) g/d).

Body composition and energy expenditure

Body composition, reported as percentage of body fat, was lower (Table 3; $P<0.001$) in both the 5 % body mass and 10 % PC groups compared with controls animals. Those animals receiving 2·5 % PC were not different from control animals. Energy expenditure, reported as V_{O2} per gram body weight per hour, did not differ from control in either treatment group.

Blood glucose and lipid chemistry

Plasma glucose concentrations on day 28 were not different across treatments (Table 4). Plasma TAG were higher ($P<0.02$) in the 5 % PC animals compared with control, but not those animals consuming 10 % PC. Total plasma cholesterol was lower ($P<0.001$) in all treatment groups compared with control animals and the response was dose dependent with 14, 38 and 53 % reductions for 2·5, 5 and 10 % PC treatments, respectively. Similarly, both HDL (45 and 52 % reductions) and non-HDL (28 and 45 % reductions)-cholesterol concentrations were lower ($P<0.001$) in groups consuming 5 and 10 % PC, respectively, compared with control animals.

Erythrocyte, liver and faecal cholesterol concentrations

There was no difference between the 2·5 % PC or controls for RBC, liver or faecal cholesterol content (Table 5).

Fractional cholesterol synthesis

Higher ($P<0.005$) cholesterol FSR was observed in the 10 % PC animals *v.* the 5 % PC animals, with only a marginally higher FSR ($P=0.08$) compared with the control animals. There were no differences between the 2·5 or 5 % supplemented group and controls (Fig. 1).

Table 3. Weight gain, body composition, food intake and oxygen consumption for Syrian golden hamsters consuming increasing concentrations of red algae (*Porphyridium cruentum* (PC)) biomass

(Mean values with their standard errors)

	Experimental diet groups							
	Control		2·5 % PC		5 % PC		10 % PC	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Weight gain (% increase)	14·2	1·6	16·5	1·5	15·9	1·6	13·2	1·5
Food intake (g/d)	8·8^a	0·2	8·5^{ab}	0·2	9·0^a	0·2	8·3^b	0·2
Body composition (% body fat)	43·6^a	1·2	48·2^a	1·1	38·8^b	1·2	38·9^b	1·1
Oxygen consumption (ml h/g)	0·92	0·05	0·75	0·05	1·00	0·06	0·94	0·05

^{a,b} Mean values with unlike superscript letters were significantly different ($P\leqq0.05$).

Table 4. Blood glucose and lipid chemistry for Syrian golden hamsters consuming increasing concentrations of red algae (*Porphyridium cruentum* (PC)) biomass

(Mean values with their standard errors)

	Experimental diet groups							
	Control		2·5 % PC		5 % PC		10 % PC	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glucose (mmol/l)	10·09	0·70	7·79	0·73	9·09	0·94	10·77	0·94
TAG (mmol/l)	2·45[a]	0·22	3·66[b]	0·28	2·49[a]	0·28	1·83[a]	0·27
Total cholesterol (mmol/l)	8·13[a]	0·32	6·89[b]	0·35	5·14[c]	0·37	3·89[d]	0·36
Non-HDL-cholesterol (mmol/l)	4·17[a]	0·30	3·56[a]	0·34	2·30[b]	0·33	1·99[b]	0·33
HDL-cholesterol (mmol/l)	3·91[a]	0·22	3·43[a]	0·21	2·80[b]	0·20	2·14[c]	0·20

[a,b,c] Mean values with unlike superscript letters were significantly different ($P \le 0.05$).

Discussion

The present primary objective was to determine whether the consumption of whole-cell biomass from PC would lower blood lipids in a dose-dependent manner in the present dietary-induced hypercholesterolaemic hamster model. Indeed, total plasma cholesterol was reduced at all three PC concentrations, 14, 38 and 53 %, respectively. There were also separate dose-dependent reductions in the level of HDL- and non-HDL-cholesterol for the 5 and 10 % PC consumption levels. Decreases in non-HDL- and HDL-cholesterol have been noted for functional foods and nutraceuticals such as plant sterols and fibres and appear to be due to efflux of cholesterol from the periphery to the liver in response to decreasing hepatic cholesterol level, not changes in LDL production[15,19]. This decrease in HDL-cholesterol, while not desirable, does not outweigh the beneficial aspect of lowering total and non-HDL-cholesterol, which is primarily LDL-cholesterol. The present findings support those of other researchers who have demonstrated a similar response in rats consuming both PC biomass and a purified polysaccharide derived from PC[13]. However, the rat does not model the human hypercholesterolaemic condition as well as the hamster. Similarly, a 28 % reduction in blood total cholesterol concentrations was reported in chickens consuming 10 % *Porphyridium* sp. for 10 d compared with controls receiving standard diet[12].

The present second objective was to address whether PC dietary supplementation reduced cholesterol synthesis rate as

the mechanism behind its ability to lower blood cholesterol. The present data for cholesterol FSR indicate that the decrease in circulating cholesterol concentrations were not due to reduced synthesis, as is the case with cholesterol lowering by statins[20]. In fact, cholesterol FSR was observed to be higher in the 10 % PC-supplemented group, most likely a compensatory increase given the lower circulating concentrations that indirectly indicate lower hepatic intracellular cholesterol concentrations. Furthermore, it has been shown recently that the regulatory controls on the processing of SREBP-2 favouring cholesterol biosynthesis are affected by very small changes in endoplasmic reticulum cholesterol concentrations[21]. Therefore, because cholesterol FSR was not decreased and actually increased at the highest levels of PC intake, we believe that the mechanism behind PC lowering of blood cholesterol is either related to a fibre-like situation of cholesterol excretion or the possibility that a particular component of the algae is affecting cholesterol transporters in favour of efflux from the liver and gastrointestinal tissues into the lumen and excreted in the faeces.

A limitation of the present study was the lack of isotopes to determine cholesterol absorption and full faecal collections to determine excretion. However, faeces and livers from

Table 5. Sterol concentrations from red blood cells (RBC), liver and faeces of hamsters consuming 2·5 % (w/w) of red algae (*Porphyridium cruentum* (PC)) biomass

(Mean values with their standard errors)

	Sterol concentrations			
	Control		2·5 % PC	
Sample	Mean	SEM	Mean	SEM
RBC (μg/g)	4·12	0·45	4·62	0·57
Liver (mg/g)	3·41	0·81	1·89	0·61
Faecal (mg/g)	5·61	0·42	6·22	0·39

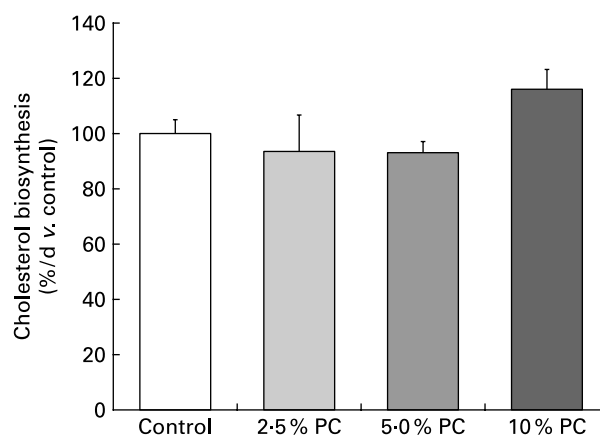RBC cholesterol *n* 13–14, liver cholesterol *n* 8–10, faecal sterols *n* 8.

Fig. 1. Cholesterol fractional synthesis (% *v.* control) from male Syrian golden hamsters with increasing doses of dietary *Porphyridium cruentum* biomass. Values are mean values with their standard errors, *n* 13–15. PC, *Porphyridium cruentum* biomass.

a subset of hamsters (2·5 % PC and controls) were collected and cholesterol content in RBC, liver and faeces was assessed. While numerically the data suggest that there is lower hepatic and higher faecal sterol concentrations, these data did not meet statistical significance. Furthermore, expressing the faecal cholesterol concentration data as mg/g *v.* total faecal cholesterol is a far less powerful measure since the high cholesterol content of the diet and total faecal output are not accounted for properly here. Therefore, our opinion regarding cholesterol excretion as the mechanism responsible for cholesterol lowering is speculative at this point.

More experiments are needed to clearly identify exact mechanisms responsible for the decrease in circulating concentrations. As mentioned previously, PC and algae in general contains high concentrations of sulphated carbohydrates, known to increase colonic mucous secretion in rats[6]. This increase in mucin production could increase faecal output and decrease intestinal transit time, thereby interfering with the recovery of cholesterol and bile acids secreted during the digestion process, much like the process associate with the hypocholesterolaemic effect of soluble fibre.

Alternatively, the composition of the algal biomass itself may shift the balance between absorption and efflux of cholesterol from both the liver and the gastrointestinal tract towards efflux and subsequent excretion. Cholesterol absorption is recognised not as a passive process but the result of a balance between the expression and the function of the known intestinal transporters, Niemann-Pick C1 Like 1 protein[22] and the ATP-binding cassette transporters (ABCG5/G8)[23]. An increase in the expression of intestinal and hepatic ABCG5/G8, target genes of the sterol-sensing nuclear hormone receptors known as liver X receptors (α and β), is a reasonable possibility, given this particular species of algae produces mammalian sterols[24,25]. Some well-known and strong agonists of the liver X receptor are oxysterols and other cholesterol precursors as well as the phytosterol, stigmasterol, each of which make up the total sterol content of PC[24,25]. Furthermore, the most potent of the endogenously produced oxysterols is 24(S),25-epoxycholesterol, which is produced at low concentrations in both human subjects and hamsters. Because human and hamster cells seem to produce higher concentrations of 27-hydroxycholesterol, a weaker agonist of the liver X receptor, they do not efficiently dispose of cholesterol through faecal excretion or storage in HDL-cholesterol when challenged with cholesterol loading, implicating this metabolism in the atherosclerotic process[26].

Overall, the data from this experiment confirm that PC as a functional component of foods reduces circulating cholesterol concentrations in a dose-dependent fashion. The present data also confirm that the mechanism by which the PC is acting to reduce the circulating cholesterol concentrations is not via reduced biosynthesis rates. While speculative, we suggest that the unique composition of PC biomass may increase faecal sterol excretion by firstly interfering with cholesterol and bile acid absorption in much the same way as soluble fibre and possibly by increasing the expression of the sterol transporter, i.e. G5/G8, in both the liver and the gastrointestinal tract collectively increasing sterol excretion.

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