Original Article

Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers

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Algal polysaccharides such as carrageenan are good sources of dietary fibre. Previous studies have shown that carrageenan has hypoglycemic effects, but its cholesterol and lipid-lowering effects have yet to be demonstrated. In this study, carrageenan was incorporated into 4 food items, then fed to 20 human volunteers to determine its effects on blood cholesterol and lipid levels. The study followed a randomized crossover design. Each phase of the study - control and experimental - lasted for 8 weeks separated by a 2-week washout. At control, the subjects consumed their usual food intake; at experimental, they were given test foods with carrageenan partly substituting similar items in their usual diet. Fasting venous blood samples were collected immediately before and after each phase to assay serum cholesterol and triglyceride. The mean serum cholesterol was significantly lower ($P < 0.0014$) after the experimental phase at 3.64 mmol/L compared with the mean level after the control phase, 5.44 mmol/L. The mean triglyceride level after the experimental phase, 0.87 mmol/L, was significantly lower ($P < 0.0006$) in comparison to the level after the control phase, 1.28 mmol/L. The mean HDL cholesterol level significantly increased ($P < 0.0071$) after the experimental phase at 1.65 mmol/L compared to the mean value after the control phase, 1.25 mmol/L. No significant differences were observed between the LDL cholesterol levels after the experimental and the control phases. This study indicates that regular inclusion of carrageenan in the diet may result in reduced blood cholesterol and lipid levels in human subjects.

Keywords: carrageenan, soluble fibre, seaweed, serum cholesterol, triglycerides, HDL cholesterol, LDL cholesterol

Introduction

Cardiovascular diseases are the foremost cause of death and the seventh cause of morbidity in the Philippines.[1] In the United States, heart disease and stroke are the first and the third causes of death, respectively.[2] Death rates due to cardiovascular diseases are also thrice greater in men 35-50 years old than in women of the same age.[3] Efforts are being made to curb the prevalence of these degenerative diseases. Dietary fibres were once known for their laxative effects. Through the pioneer work of Burkitt and Trowell in the 1960's, these were introduced to the medical world and became important in the field of human nutrition. From the 1960's to the present, researchers have found that soluble dietary fibres are negatively associated with cardiovascular diseases such as hypertension and coronary heart disease, gastrointestinal diseases, obesity and cancers.[4]

Seaweed extracts, such as carrageenan, are one of the sources of soluble dietary fibre. Carrageenan is an algal polysaccharide that is extracted by water or aqueous alkali from dried Eucheuma.[5] It is widely used as a gellant, stabilizer, thickener and texture modifier in the food industry. Carrageenan may also be used to control the cholesterol content in foods because of its ability to mimic the texture and sensory qualities of fat, reducing the total amount of fat in food.[6]

Previous studies[7,8] showed that carrageenan incorporated into common Philippine foods such as fishballs and *arroz caldo* has hypoglycemic effects in normal subjects. A study done on another seaweed extract showed that when incorporated into *puto*, *siomai* and a meal composed of rice and meatballs with sweet-and-sour sauce, agar had a glucose-lowering effect in normal subjects.[9] However, the effects of carrageenan on blood cholesterol and lipid levels were unknown prior to this investigation. This study aimed to determine the effects of carrageenan, incorporated into locally consumed foods, on the blood cholesterol and lipid levels of free-living human volunteers.

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Materials and methods

Experimental Food Products

Four experimental food products were used in the menu of the subjects - *pan de sal, maja blanca, arroz caldo* and fishball. *Pan de sal* is a yeast bread popular in the Philippines. Maja blanca is a corn pudding eaten as dessert or snack. *Arroz caldo* is a gruel-like mixture of rice, chicken meat, ginger and other spices. Fishball is a fried dumpling-like blend of fish and binders dipped in sauce.

Carrageenan in powder form was incorporated into the mixture of ingredients before cooking, being hydrated in the process. Cooking methods were standardized in a previous study by the researchers.[6,9] At roughly 37.5±2.5 g a piece, two pieces of *pan de sal* were given to each subject for breakfast and three pieces for morning and afternoon snacks. Two 80g portions of *maja blanca* were given daily as dessert. *Arroz caldo* and fishballs were given occasionally, depending on the preference of the subjects. *Arroz caldo* was packed in 200g portions while fishballs, at 13g per ball, were packed by three. A pack of fishballs was given whenever a subject called for its inclusion in his diet.

The subjects' weighed and labelled food supplies were placed in microwavable containers and frozen before distribution. *Pan de sal, arroz caldo* and fishballs were thawed and reheated in a microwave oven (Goldstar Multiwave® MA-6915-A) before consumption. Maja blanca was thawed to chilling temperature. The subjects' intake of the experimental food items were monitored and recorded daily. Food items were combined such that the diet of each subject provided approximately 40 grams dietary fibre a day.

Subjects

The protocol of the study was approved by the ethics committee of the funding agency. It was conducted in accordance with the internationally agreed ethical principles for the conduct of medical research. Twenty (4 males and 16 females) randomly selected subjects from the University of the Philippines aged 28-61 years participated in the study after signing informed consent. It was emphasized that no lifestyle and dietary modifications should take place within the study period. Subjects were weighed every 10 days at each phase of the study.

In-vivo Test

The study followed a randomized crossover design, wherein subjects served as their own control. Each subject participated in both phases of the experiment - control and experimental, each period lasting for eight weeks separated by a two-week washout. Before the subjects were given their phase assignment, pre-study data on their weight and height were gathered and a 3-day 24-hour food recall was conducted. Subjects' weights were obtained using a calibrated balance scale (Detecto) and height with the aid of a microtoise. The subjects' baseline characteristics are shown in Table 1.

Subjects assigned to the control phase consumed their regular diet. On the other hand, subjects on the experimental phase were given test foods to substitute part of their regular diet based on the analysis of the test foods' major components (Table 2). This was to ensure that the control and experimental diets had similar amounts of available carbohydrates, fats and protein.

During the study, a 24-hour recall was conducted every ten days. Food recall data were analyzed using the Bender & Strands Nutrition Software. The Philippine Composition Tables (1997) and Bowes and Church's Food Values of Portions Commonly Used were consulted to compute food items not found in the database.

Venous blood was extracted immediately before and after each phase after a 10- to 12-h fast. Blood samples were drawn between 6 and 8 o'clock in the morning at the laboratory of the University of the Philippines Health Service with the aid of medical technologists. Analyses for cholesterol and triglyceride levels were done within 1h of obtaining the blood samples.

Table 1. Baseline characteristics of subjects (N = 20)

Variable	Range	Mean ± SD†
Age (yr)	28 - 61	41 ± 8.89
BW‡ (kg)	40.57 - 72.73	60.83 ± 12.54
Height (m)	1.45 – 1.66	1.55 ± 0.05
BMI§ (kg/m²)	16.6 - 34.7	25.1 ± 4.39

†Standard deviation; ‡Body Weight; § Body Mass Index

Table 2. Proximate composition (% wet basis) of the test foods†

Components	*Arroz Caldo*	*Maja Blanca*	*Pan de Sal*	*Fishball*
Moisture	86.65 ± 0.33	76.12 ± 0.11	41.20 ± 0.13	51.75 ± 0.37
Crude protein	0.67 ± 0.07	0.67 ± 0.23	8.49 ± 1.27	12.46 ± 0.41
Crude fat	0.54 ± 0.13	2.04 ± 0.35	1.26 ± 0.04	8.25 ± 0.11
Available carbohydrate	8.19 ± 0.31	13.09 ± 0.15	29.16 ± 0.89	10.62 ± 0.37
Total dietary fibre	2.03 ± 0.13	8.01 ± 0.15	18.38 ± 0.11	14.10 ± 0.14
Ash	1.51 ± 0.07	0.99 ± 0.08	1.19 ± 0.03	2.81 ± 0.15

†Values are means and standard deviations of 9 replicates except for total dietary fibre where there were only 3 replicates

Blood Analysis

Collected blood samples were centrifuged (Digisystem Laboratory Instruments, Inc.) for 15 min at 6,000 rpm to separate the serum. In a 12x75 mm test tube, 10 µL serum was mixed with 1 mL cholesterol reagent (Cholesterol FS, DiaSys Diagnostic Systems GmbH & Co., Germany).

Similarly, 10 µL serum was mixed with 1 mL triglyceride reagent (Dialine Diagnostic System, Germany). These were incubated at 37°C for 5 and 10 min for cholesterol and triglyceride analysis, respectively. Serum cholesterol and triglyceride were measured using Premier Plus Stanbio Analyzer (Stanbio Laboratory, Texas, USA) with photometric accuracy of ±1% of the reading +0.005 Abs.

Precipitation technique was specifically indicated for HDL cholesterol determination. Five hundred µL precipitant (Dialine Diagnostic System, Germany) was made to react with 200 µL of the serum, which was allowed to stand for 10 min. The mixture was then centrifuged for 15 min at 5,000 rpm, then 100 µL supernatant was mixed with the cholesterol reagent (Cholesterol FS, DiaSys Diagnostic Systems GmbH & Co., Germany), then incubated for 5 min. HDL cholesterol was also determined using the Premiere Plus Stanbio Analyzer. As for LDL cholesterol, the previously determined serum cholesterol and lipid components were used in calculating its level. The formula used (Dialine Diagnostic System, Germany) was: LDL cholesterol (mmol/L) = total cholesterol – HDL cholesterol – [Triglycerides / 2.2]

Statistical Analysis

Results are reported as mean ± SD. Regression analysis using the Statistical Analysis Software (SAS ver. 8.01 1999-2000, Cary, N.C.) was used to test differences in lipid response due to treatment as well as nutrient intake. The adjusted mean for lipid and cholesterol levels were also calculated using the model derived from regression analysis. This was used to correct for heterogeneity caused by differences in baseline values and the carryover effect usually observed in experiments with crossover designs.

Table 3. Mean body weight of subjects at control and at experimental phases (N = 20)

Weight (kg)	Control phase		Experimental phase	
	Week 0	Week 8	Week 0	Week 8
Mean ± SD†	60.33 ± 12.06	61.11 ± 12.33	60.84 ± 12.42	60.49 ± 11.88

†Standard deviation

Results

Weight Change

The mean body weights of subjects at control and at experimental phases are shown in Table 3. It may be noticed that during the control phase, the mean body weight slightly increased from baseline until after eight weeks. On the other hand, the subjects' mean body weight during the experimental phase slightly decreased from the baseline after eight weeks. However, these changes were not significantly different across phases.

Dietary Intakes

Table 4 shows the mean nutrient intake of the subjects during the two phases. Calories, fat and carbohydrates were slightly higher during the experimental phase but not significantly different from the control phase. Likewise, protein, calcium, beta-carotene, niacin, cholesterol and sodium intakes of the subjects were not significantly different between the two phases. However, the subjects' mean intakes of iron, thiamin, riboflavin and total dietary fibre were significantly higher during the experimental compared to the control phase.

Table 4. Least squares estimates of the mean nutrient intake of subjects (N = 20) at control and at experimental phases.

Nutrient	Control	Experimental
Calorie (Kcal)	1685	1881
Protein (g)	58.2	67.4
Fat (g)	40.1	38.7
Carbohydrate (g)	273.1	315.8
Calcium (mg)	598	492
Iron (g)*	14.1	18.3
Beta-carotene (µg)	1119	1474
Thiamin (mg)**	0.67	1.00
Riboflavin (mg)***	0.76	1.18
Niacin (mg)	14.0	17.3
Vitamin C (mg)	41	47
Cholesterol (mg)	140	144
Sodium (mg)	3185	3322
Total dietary fibre (g)****	10.7	39.9

Statistically significant *$P < 0.0181$; **$P < 0.0021$; ***$P < 0.0011$ ****$P < 0.0001$

Cholesterol and Lipid Levels

Serum Cholesterol

The mean value for total cholesterol was significantly lower ($P<0.0014$) at 3.64 mmol/L after eight weeks of consumption of the test diet compared to the corresponding value at the control phase, 5.44 mmol/L, after eight weeks of consumption of their usual diet (Fig. 1). The mean difference was 1.80 mmol/L or 33%.

Serum Triglycerides

The subjects' mean serum triglyceride level after eight weeks under experimental phase was also significantly lower ($P< 0.0006$) at 0.87 mmol/L compared with the corresponding level after eight weeks under the control phase, 1.28 mmol/L. The mean difference was 1.20 mmol/L or 32% (Fig 2).

HDL Cholesterol

The mean HDL cholesterol of the subjects after 8 weeks at the experimental phase was 1.65 mmol/L, which was significantly higher ($P < 0.0071$) than the level obtained just after the control phase, 1.25 mmol/L. The mean difference was 0.40 mmol/L or 32% (Fig 3).

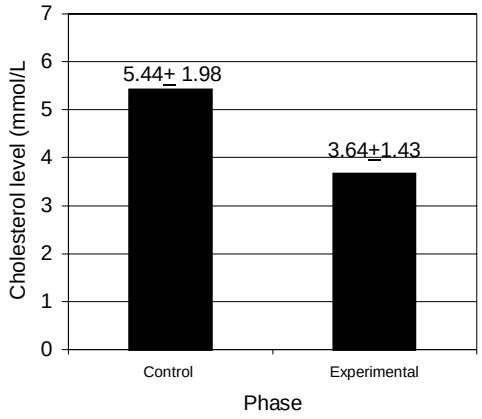

Figure 1. Mean serum cholesterol levels of subjects (N = 20) at control and at experimental phases. Levels are significantly different at $P < 0.0014$.

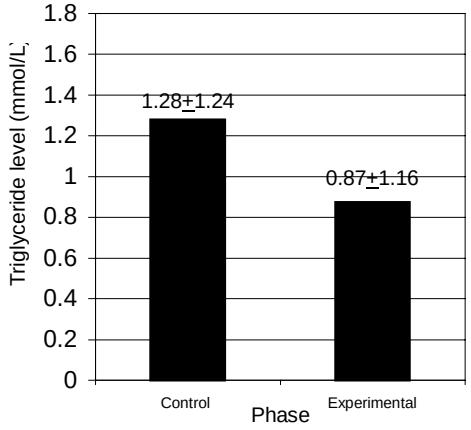

Figure 2. Mean serum triglyceride levels of subjects (N = 20) at control and at experimental phases. Levels are significantly different at $P < 0.0006$.

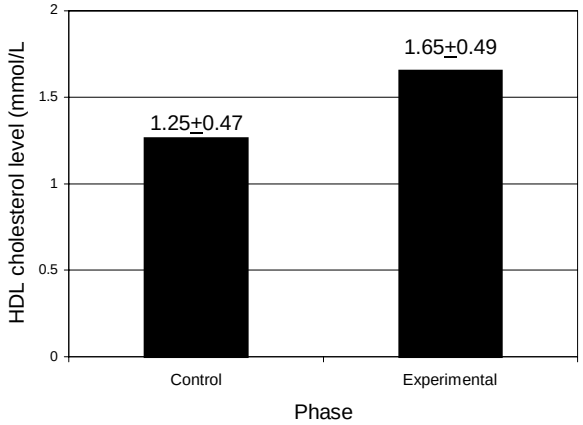

Figure 3. Mean serum HDL cholesterol levels of subjects (N = 20) at control and at experimental phases. Levels are significantly different at $P < 0.0071$.

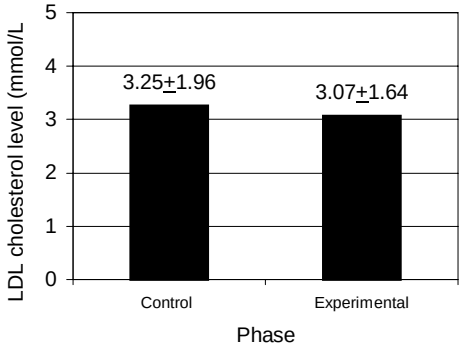

Figure 4. Mean serum LDL cholesterol levels of subjects (N = 20) at control and at experimental phases. Levels are not significantly different.

LDL Cholesterol

The subjects' mean LDL cholesterol level immediately after the experimental phase was lower (3.07mmol/L ± 1.64) than the corresponding level just after the control phase (3.25 mmol/L ± 1.96) (Fig 4). However, the values did not vary significantly.

Discussion

Weight Change

The absence of significant difference in weight at control and at experimental phases indicates that whatever differences there were in the cholesterol and lipid levels were due to the effect of fibre. This observation is an important aspect of the study, for weight loss has been correlated to changes in serum lipid levels as shown in the studies of Andersen *et al.,* and Dattilo and Khris-Etherton.[10,11]

Dietary intake

Dietary components that are known to influence cholesterol levels include energy intake and fibre (Khris-Etherton *et al.*, 1988). Niacin, on the other hand, has been shown to decrease cholesterol levels. However, in the present study, the intake of calories, carbohydrate, fat, protein, calcium, beta-carotene, niacin, cholesterol and sodium during the control and the experimental phases were not significantly different. Therefore, whatever differences there were in the subjects' serum cholesterol and lipid levels may be attributable to the significantly higher amount of fibre in their diet.

Blood cholesterol and lipid levels

Aside from weight changes, alterations in dietary intake could also serve as variables in determining the independent effects of soluble fibre. In the present study, the calorie, carbohydrate, fat, protein, calcium, beta–carotene, niacin, cholesterol and sodium intake of the subjects were not significantly different in both phases. However, the subjects' mean intakes of iron, thiamin, riboflavin and total dietary fibre were significantly higher during the experimental compared to the control phase. Considering this, the significant changes in the

cholesterol and lipid levels of the subjects as seen in the present study could then be attributed independently to the increased fibre intake of the subjects during the experimental phase.

Serum Cholesterol

The result of the present study on serum cholesterol is similar to those obtained in other studies. In the study of Anderson *et al.,* on the effect of oat-bran, they observed that total cholesterol level was 12.8% lower ($P < 0.01$) after the 21 day oat-bran diet compared to the level at the control phase.[12] In the study by Poulter and co-workers, small but significant decreases in total cholesterol were observed (2.23%, $P<0.04$) after 4 weeks of consuming an oat-based cereal compared to the usual-cereal containing no oats.[13] Similarly, Panlasigui and Tiangson noted that total cholesterol level after 2 months of oatmeal consumption was significantly lower by 5.5% ($P<0.0348$) compared to the level at control phase.[14]

Some other sources of fibre were tested in other studies. In the study of Nicolosi *et al.*, a 6% decline ($P<0.05$) from baseline in cholesterol level was observed after 8 weeks using beta-glucan from yeast as the source of soluble fibre.[15] In the study by Landin *et al.*, total cholesterol level after the guar gum treatment was lower by 7% compared to the level after the placebo treatment.[16] The study of Markkola *et al.,* on the other hand showed that total cholesterol level fell by 16% after 3 weeks during the guar gum treatment.[17] In the study of Davidson *et al.,* using cereal enriched with psyllium on children with hypercholesterolemia, cholesterol level decreased by 5% ($P<0.03$) from baseline during the psyllium phase, whereas no significant change was observed during the placebo phase.[18] The results of the mentioned studies on oats, beta-glucan, guar gum and psyllium in terms of effects on cholesterol levels were identical to the results observed in this study. However, the observed decrease in total cholesterol, compared to the level at the control phase, was highest in the present study at 33% compared to those observed in the previously mentioned studies, where a range of 2-16% decrease in total cholesterol was observed**.**

Serum Triglyceride

In terms of serum triglyceride changes, the result of the research by Landin *et al.,* using guar gum[16] is similar to what was obtained in the present study. The former showed a 15% decrease ($P < 0.05$) in serum triglycerides after the guar gum treatment compared to the placebo. This observed decrease in serum triglyceride is lower compared to the observed decrease in the present study (32%). However, some studies do not support the triglyceride-lowering effect of soluble fibres. In the study by Anderson *et al.*, a 10% reduction in serum triglycerides was observed after both the 21 day oat-bran and wheat-bran diet compared to the control phase, but only the latter showed significant change.[12] In other studies,[14,15,17,18] serum triglyceride was reduced during the experimental phase (using oatmeal, beta-glucan from yeast, guar gum and psyllium, respectively) but the changes were not significantly different when compared to those obtained during the control or placebo phase. In contrast, Poulter and co-workers observed that serum triglycerides increased by 11.86% relative to the control phase or the usual-cereal with no oats phase, though the change was not significant.[13]

HDL Cholesterol

The increase in HDL cholesterol level as observed in the present study was similarly noted by Nicolosi *et al.*[15] In their investigation of the effects of intake of beta-glucan fibre from yeast for eight weeks, HDL cholesterol increased significantly by 9% ($P<0.005$). The researchers also observed a 16% significant increase ($P< 0.05$) in HDL cholesterol level four weeks after fibre supplementation was stopped, which indicates carry-over effects of soluble fibre from beta-glucan on HDL cholesterol. In the present study, the increase (32%) was higher than that observed in the study by Nicolosi and coworkers.

There are other studies that have shown decreases in HDL cholesterol levels after soluble fibre supplementation, though these decreases were not significant. Such was observed in the study of Anderson *et al*., where there was a 7.4% decrease in HDL cholesterol after the 21-day oat-bran diet.[12] In the study of Poulter *et al.*, HDL cholesterol fell by 2.69% after consumption of oat-based cereal relative to the usual-cereal consumption.[13] In the study of Davidson *et al.*, HDL cholesterol level decreased by about 2% after consumption of psyllium-added cereals.[18] HDL cholesterol level remained unchanged after the guar gum consumption in the study of Markkola *et al.*[17] However, in the study by Panlasigui and Tiangson, the decrease in HDL cholesterol level was significant ($P<0.051$), with the level during the control phase higher by 19.5% compared to the level during the experimental phase.[14]

LDL Cholesterol

Most studies on soluble fibre and lipid changes show that changes in LDL cholesterol are parallel to the changes in serum cholesterol. In the study by Anderson and coworkers, the LDL cholesterol level of subjects decreased by 12.1% ($P < 0.01$) after the oat-bran diet.[12] In the study by Poulter *et al.*, small but significant decrease (4.55%, $P<0.05$) in LDL cholesterol level was observed after consumption of the oat-based cereal relative to the usual-cereal consumption.[13] In the study by Nicolosi *et al.*, an 8% decrease in LDL cholesterol concentration from baseline or control phase was observed after 8 weeks.[15] In Markkola's study, a 20% decrease in LDL cholesterol level was observed after the guar gum consumption.[17] The result of this study in terms of LDL cholesterol level does not agree with the results of the previously mentioned investigations. Despite significant changes in total cholesterol levels (33% lower at experimental phase compared to the level at control phase), the decrease in LDL cholesterol level (6%) was not significant. This may be due to high variability among individual values, as reflected in the standard deviation.

How fibre exerts its hypocholesterolaemic effects is correlated with its ability to bind cholesterol-rich bile in the lower small intestines, resulting in lesser cholesterol

absorbed. With the binding of bile by dietary fibres, less bile is reabsorbed. Serum cholesterol further decreases as cholesterol is used by the liver to replenish the bile acid pool.[19] Fermentation of dietary fibre in the large intestines also decreases the biosynthesis of cholesterol. Propionate, one of the short chain fatty acids produced by the fermentation of dietary fibres, inhibits the rate by which the liver produces the body's cholesterol.[19]

In the present study, carrageenan, being the main source of dietary fibre, may have brought about hypocholesterolaemic effects due to its ability to bind bile acids and cholesterol in the lower small intestines. With the binding of bile, lesser cholesterol is absorbed. Since bile is made from cholesterol, circulating cholesterol further decreases since it is used to compensate for the lost bile.

Reduction of serum cholesterol and lipids may also be due to changes in the physical properties of the intestinal contents related to the ability of fibres to provide bulk, volume and viscosity of the contents - all of which are important in slowing the rate of digestion and absorption of nutrients. Dietary fibres provide bulk since they are not digested, so they remain during the transit of the digesta through the small intestines. The water-holding capacity of carrageenan results in increased volume of the intestinal contents. On the other hand, viscosity of the intestinal contents increases due to the presence of viscous polysaccharides such as carrageenan.

The increased bulk and viscosity of the intestinal contents and the decreased rate of digestion and absorption also results in slowing down of the diffusion of enzymes, substrates and nutrients to the absorptive phase, which then results in the lower levels of nutrients, including cholesterol, after a meal, manifested as hypocholesterolemic effect.[20]

It is concluded that carrageenan incorporated in locally consumed foods such as *pan de sal*, *maja blanca,* fishball, and *arroz caldo* significantly reduces the blood cholesterol and lipid levels in free-living human volunteers.

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