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Effects of diet supplementation with three soluble polysaccharides on serum lipid levels of hypercholesterolemic rats

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Abstract

Some soluble polysaccharides have been explored as possible ingredients in the development of ''functional foods'' because of their ability to reduce plasma cholesterol and consequently contribute to the reduction of the risk of cardiovascular disease. However, this effect has been predominantly observed in studies using megadoses that are incompatible with the pattern of human consumption. On the basis of this limitation, the objective of the present study was to assess the hypocholesterolemic effect of the ingestion of three different hydrocolloids (guar gum, xanthan gum and pre-gelatinised cornstarch) at 1.5% concentration in an assay on rats. Cellulose was used as control. In the first stage of the experiment, all animals were fed a hypercholesterolemic diet that produced a mean concentration of total cholesterol (5.2 mmol/l) significantly higher than in the group fed a control diet (1.0 mmol/l). In the subsequent stage, 1.5% of the starch was replaced with each of the three hydrocolloids in the experimental ration. Food intake and body weight gain were not altered by the hydrocolloid diets ($P<0.05$). Serum cholesterol concentrations were 1.43, 1.41, 1.54 and 1.42 mmol/l for the diets containing guar gum, xanthan gum, pre-gelatinized cornstarch and cellulose, respectively. We conclude that, under the conditions of the present study, the hypocholesterolemic effect of hydrocolloid ingestion at a dose of 1.5% of the ration was not significant in comparison to the effect produced by the reduction and change of lipids in the diet. Thus, more attention should be paid to the use of hydrocolloids in functional foods, always considering the balance of the diet as a whole.

Keywords: Hydrocolloids; Functional foods; Serum cholesterol; Soluble fibres

1. Introduction

Although each country has a specific definition, any food that exerts a positive impact on health, in addition to its natural nutritional contribution, might be considered a ''functional food'' (Kwak & Jukes, 2001). The world market for this type of food is currently growing, with sales estimates of the order of US$ 60 billion, mainly as a consequence of greater consumer consciousness regarding the importance of the diet for life quality (Hardy, 2000; Hilliam, 1996; Swadling, 2001). The development of a functional food explores the relationship between the ingestion of a certain nutrient and the reduced risk of a specific disease or improved performance. One of the most extensively discussed dietary functional relationships is that between the ingestion of some soluble polysaccharides, especially those rich in soluble fibres, and the reduced risk of coronary disease (Roberfroid, 1999).

Various epidemiological studies have shown an association between higher soluble fibre intake and a reduced risk of coronary disease due to a reduction in total plasma cholesterol concentration, although the physiological mechanism explaining the hypocholesterolemic effect of these fibres is not completely understood. The most widely discussed hypotheses include the interference with lipid absorption and metabolism, impaired cholesterol absorption, increased excretion of bile acids and sterols, altered cholesterol synthesis or accelerated uptake of lipoprotein by the liver, production of short-chain fatty acids from fibre fermentation in the colon, alterations in the concentration or sensitivity to insulin or other hormones, and increased viscosity of the gastrointestinal contents, which reduces the gastric emptying rate and contributes to a sensation of satiety. However, the potential hypocholesterolemic effect of these hydrocolloids is limited by the amount of hydrocolloids ingested with the human diet due to the sensory characteristics of these compounds (Anderson, Deakins, Floore, Smith, & Whitis, 1990; Anderson, Jones, & Riddell-Mason, 1994; Hosobuchi, Rutanassee, Bassin, & Wong, 1999;

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Nomenclature

HYPER	groups receiving a hypercholesterolemic diet
CONT	a control AIN93M diet
GUAR	diets containing guar gum
XANT	xanthan gum
PGCS	pre-gelatinized cornstarch
CELL	cellulose

Levrat-Verny, Behr, Mustad, Rémésy, & Demigné, 2000; Malkki et al., 1993; Overton et al., 1994).

Guar gum is a galactomannan extracted from the endosperm of the Indian cluster bean (*Cyanopsis tetragonoloba* L.). Xanthan gum is a biosynthetic edible gum produced by the bacterium *Xanthomonas campestris*, and consists of glucose, mannose and glucuronic acid. These polysaccharides are used in different foods as thickeners and stabilizers. Xanthan dispersions submitted to heating or cooling, do not show alterations in the viscosity of the solution which leads to a broad applicability of this hydrocolloid in foods. Modified cornstarches, such as pre-gelatinized starch, present functional properties similar to some soluble fibres and they have been used for thickening with success (Levrat-Verny et al., 2000; Morris, 1995; Rayment, Ross-Murphy, & Ellis, 2000). Based on the capacity of some soluble polysaccharides to form solutions of high viscosity at low temperatures, even when applied at low concentrations, the objective of the present study was to determine alterations in serum total cholesterol and triacylglycerol concentrations in rats submitted to diets containing three different soluble polysaccharides (guar gum, xanthan gum and pre-gelatinized cornstarch), widely used as thickeners in human food formulations.

2. Materials and methods

2.1. General

The percentage compositions and soluble and insoluble fibre contents of guar gum (Colloides Naturels Intern.), xanthan gum (Archer Daniels Midland Co.), pre-gelatinized corn starch (National Starch & Chemical Ltd.) and cellulose fibrous medium (C6288-Sigma Chemical Co. St. Louis, MO) were determined by conventional methods according to AOAC (1990).

2.2. Experimental design and diets

The present study was divided into two phases, each lasting 26 days. During the first phase, the animals were rendered hypercholesterolemic by receiving a diet containing 25% saturated fat, 1% cholesterol, and 0.1% cholic acid (hypercholesterolemic diet), as described by Zulet, Barber, Garcin, Higueret, and Martinez (1999). During the second phase, animals received a diet prepared according to the AIN93M (Reeves, Nielsen, & Fahey, 1993), with the 1.5% (w/w) corn starch being replaced with 1.5% guar gum, xanthan gum, pre-gelatinized corn starch or cellulose. Insoluble fibres such as cellulose have no expressively hypolipidemic action and their application has been suggested as a "control" in animal and human studies (Anderson et al., 1994). The proportion of 1.5% of each hydrocolloid in the experimental diets was based on the estimated calorie intake of rats (70 kcal/day) compared to humans (2500 kcal/day) obtained in a previous pilot study, corresponding to a daily intake of approximately 10 g hydrocolloid in the human diet, equivalent to 0.4 g/100 kcal. The composition of the diets is shown in Table 1.

2.3. Animal experiments

Adult male Wistar rats obtained from the Biological Sciences Institute, University of São Paulo, were housed in individual stainless-steel cages and fed standard rat diet (AIN93M) for two days for adaptation. The animal room was adjusted to a temperature of 22 ± 2 °C, relative

Table 1
Composition of the diets

Ingredients[a] (g/100g)	Control[b]	Hypercholesterolemic[c]	Test
Casein	14.00	14.000	14.00
DL Methionine	0.18	0.180	0.18
Corn Starch	46.57	29.06	45.07
Dextrin	15.50	15.50	15.50
Vitamin Mix	1.00	1.00	1.00
Mineral Mix	3.50	3.50	3.50
Choline	0.25	0.25	0.25
Sucrose	10.00	10.00	10.00
Cellulose	5.00	–	5.00
Hydrocolloids[d]	–	–	1.50
BHA (mg)	0.80	5.20	0.80
Soy Óil	4.00	–	4.00
Coconut oil	–	25.00	–
Cholic acid	–	0.50	–
Cholesterol	–	1.00	–
Total	100.00	100.00	100.00

[a] Casein (Sigma C7078), DL-Methionine (Sigma M9500), corn starch and dextrin (Corn Products Brazil Ing Ind Ltda), Vitamin and Mineral Mix according Reeves et al. (1993) to adults animals, choline bitartrate (Sigma C1629), cellulose fibrous medium (Sigma C6288), BHT (Ionol/ Butilhidroxitolueno/Synth), Coconut Oil (Coconut Oil Sigma C-1758) Cholic acid (Sigma C1129) and cholesterol (5-cholesten- 3β-cholon-24-oic acid/ Sigma C3292).

[b] Control: AIN-93M Reeves et al. (1993).

[c] Hypercholesterolemic: modificated from Zulet et al. (1999).

[d] Hydrocolloids: Guar gum (Colloides Naturels International), Xanthan gum (Archer Daniels Midland Co.) and pre-gelatinized corn starch (National Starch & Chemical Ltda.).

humidity of 55±10%, a light-dark cycle of 12h. For the experiment, animals weighing 193–207 g were divided into six groups, with approximately the same weight distribution in each group and received powdered diets and water ad libitum. Food consumption and fecal volume were recorded three times a week and the animals were weighed individually once a week. Feces were weighed after drying in an incubator at 105 °C. During the first phase of the experiment (days 1–26), all groups received the hypercholesterolemic diet (HYPER[1–5]), except for the control (CONT) group which received the AIN93M diet (Reeves et al., 1993). At the end of this period, the animals were fasted for approximately 16h, sedated with ether, anesthetized with non-vasoconstrictive lidocaine and submitted to caudal puncture for blood collection (500 to 1000 μl) into polyethylene tubes prewashed with heparin (4×10^5 U/l; Sigma Chemical Co.) according to Yaniv, Schaffermann, Shamir, and Mader (1999). The samples were centrifuged for 4–5 min at 10,000 *g*, and total cholesterol and triacylglycerol were immediately determined in the serum. The animals were then submitted to the second phase of the experiment (days 27–52), during which one group continued to receive the hypercholesterolemic diet (HYPER), while the other four groups received diets containing guar gum (GUAR), xanthan gum (XANT), pre-gelatinized corn starch (PGCS) or cellulose (CELL). At the end of this period, the animals were fasted for approximately 16 h and killed by decapitation. Blood samples were collected (5–7 ml), centrifuged and analyzed as described for the previous phase and the liver was immediately removed and weighed.

2.4. Serum measurements

Total cholesterol and triacylglycerol levels were determined by colorimetric enzymatic methods using commercial kits purchased from Bayer (Bayer Sera-Pak Fast Color Nos. 6670/6671 and 6684/6687). All samples were analyzed within a single batch for each phase.

2.5. Fibre analysis

Total fibre content of the soluble polysaccharides and of the total diets were determined using an enzymatic-gravimetric method (AOAC, 1990), as modified by Prosky, Asp, Schweizer, de Vries, and Furda (1992). Quadruplicate samples (1 g for cellulose and starch, and 200 mg for guar gum, xanthan gum and diets) were digested with thermostable alpha-amylase at pH 6.0, protease at pH 7.5, and amyloglucosidase at pH 4.3 to remove protein and starch. The hydrolysate was vacuum filtered through crucibles pre-washed with dextran solution and sintered with glass wool to separate the soluble and insoluble fractions. Four volumes of 98% ethanol were then added to precipitate soluble dietary fibres. The residue was filtered and washed with 78% ethanol, 95% ethanol and acetone. After drying, the residue was weighed and analyzed in duplicate. One sample was used for protein determination and the other was incinerated at 525 °C for ash determination.

$$\%\ \text{Insoluble Fibre (IF)} = [(\text{IR} - P - A - W')/W]\times 100$$

where IR = Insoluble Residue of the sample (mg), P = protein of the IR (mg), A = ash of the IR (mg), W' = white (IR), W = weight of the samples (mg).

$$\%\ \text{Soluble Fibre (SF)} = [(\text{SR} - P - A - W')/W] \times 100$$

where

SR = Soluble Residue of the sample (mg), P = protein of the SR (mg), A = ashes of the SR (mg), W' = white (SR), W = weight of the samples (mg).

2.6. Fatty acid analysis

The lipid fraction was extracted from the diet samples as described by Folch, Lees, Sloanne, and Stanley (1957). Fatty acid esterification was obtained according to Hartman and Lago (1973). The solutions were injected into a gas chromatograph (GC17A Shimadzu Class CG) equipped with a 10 m×0.25 mm (inner diameter) fused silica capillary column (Supelcowax) and a flame ionization detector. Helium was used as the carrier gas and the fatty acids were separated at a temperature gradient ranging from 80 to 150 °C (6°C/min). Fatty acids were calculated from the peak areas relative to the peak area of the internal standard.

2.7. Statistical analysis

Two-way analysis of variance (MANOVA) was performed and specific group comparisons were made using the Tukey test (Bower, 1998), where P values of <0.05 were taken as statistically significant. Data analysis was carried out using the STATISTICA/6.0 program (STATISTICA Inc. Tulsa, USA, 2002).

3. Results

The soluble and insoluble fibre contents of each polysaccharide evaluated in the present study is shown in Table 2. Cellulose was used as a control in the experimental assays because of its high insoluble fibre content. Although pre-gelatinized cornstarch is solubilized at low temperatures, forming a high viscosity solution like guar and xanthan gums, this compound was completely digested by alpha-amylase and amyloglucosidase. The nutritional composition of the diets used in the experiments confirmed the high concentration of total lipid

Table 2
Dietary fibres of the soluble polysaccharides (g/100 g)

Dietetic fibre[a]	Cellulose	Guar gum	Xanthan gum	Pre-gelatinized corn starch
Soluble fibre	0.37	61.81	45.92	0.00
Insoluble fibre	94.28	11.80	13.71	0.00
Total fibre	94.65	73.61	59.63	0.00

[a] Values are means ($n=4$).

and saturated fatty acids in the hypercholesterolemic diet (Table 3) and the proportions of soluble and insoluble fibres in each experimental diet (Table 4).

Body weight, food intake, feces volume, and serum total cholesterol and triacylglycerol concentration during the first phase (days 1–26) are shown in Table 5. Mean weight gain was 52% higher in the HYPER$^{1-5}$ groups (2.4 g/day) than the CONT group (1.6 g/day), although a weight gain similar to that of the CONT group (1.9 g/day was observed for one group (HYPER^{1}). With respect to food intake during the first phase, the HYPER$^{1-5}$ groups showed a 22.3% lower mean total food intake (377 g) than the CONT group (485 g). The lower food intake associated with an equal or higher weight gain is due to the high energetic value of the hypercholesterolemic diet (492 kcal) compared to the control diet (361 kcal). The fecal volume of the CONT group was 43% larger than that of the other groups, indicating both a higher food intake and a more equilibrated diet composition in terms of lipid and fibre concentration (Table 3).

The consumption of the diet containing 25% of saturated fat during the first 26 days of the experiment was efficient in producing hypercholesterolemia and hypertriacylglycerolemia in the animals, provoking a significant increase in both serum total cholesterol (5.2 and 1.0 mmol/l) and triacylglycerol (4.5 and 3.1 mmol/l) in the HYPER$^{1-5}$ and CONT groups, respectively. This efficacy was achieved because energy restriction was not observed (Nomani et al., 2000).

Table 6 shows the weight gain, food intake, feces volume, serum total cholesterol and triacylglycerol concentration, and relative liver weight observed during the second phase (days 26–52). The CONT group showed a 37% higher weight gain than the other groups, with no significant difference in weight gain in these other groups. The difference between the CONT and experimental groups may be due to the effect of the sudden alteration in the diet of HYPER$^{1-5}$ animals on the natural weight gain of these animals. With respect to food intake, the HYPER group ingested a lower amount than the other groups due to the higher calorie content of the diet and general misbalance. GUAR and PGCS groups showed a food intake similar to that of the CONT group. Food intake was slightly higher in XANT and CELL animals. However, no significant difference in food intake was observed between the four experimental groups. Levrat-Verny et al. (2000) also did not observe any difference in food intake or weight gain of rats fed a diet supplemented with 1% guar or xanthan gum. Fecal volume was significantly higher in the XANT group than in the other groups. The colour and texture of the feces were also altered in this group. Fecal volume was lower in the HYPER group, with moisture content (29.7%) being almost twice as high as in the other groups (on average

Table 3
Nutritional composition of the control and hypercholesterolemic diets (g/100 g)

Nutrients[a]	Control	Hypercholesterolemic
Moisture	7.5±0.4	5.3±0.1
Protein (Nx6.25)	13.2±0.3	13.5±0.2
Carbohydrate[b]	67.4	54.4
Lipid	4.3±0.2	24.5±0.3
Monounsaturated fatty acids[c]	35.4±0.10	1.8±0.01
Polyunsaturated fatty acids[c]	58.4±0.17	6.9±0.03
Saturated fatty acids[c]	4.7±0.02	91.2±0.03
16:1	11.0±0.02	–
18:1	24.3±0.04	6.92±0.03
20:1	0.21±0.01	–
18:2	53.3±0.17	1.82±0.01
18:3	5.11±0.00	–
06:0	–	0.30±0.01
08:0	–	7.06±0.15
10:0	–	5.81±0.05
12:0	–	48.1±0.07
14:0	0.07±0.01	18.2±0.10
16:0	0.10±0.01	9.01±0.04
17:0	–	–
18:0	3.53±0.01	2.78±0.02
20:0	0.38±0.01	–
22:0	0.46±0.01	–
24:0	0.16±0.00	–
Not identified[c]	1.38±0.16	–
Ashes	2.9±0.1	2.3±0.0
Dietetic fibre	4.7	0.0
Energy (kcal)	361.1	492.1

[a] Values are means±SD ($n=3$).
[b] Values obtained by difference.
[c] Values are% means of total lipids ($n=3$).

Table 4
Nutritional values of experimental diets

Nutrients[a] (g/100 g)	GUAR	XANT	PGCS
Moisture	7.0±0.2	6.6±0.1	6.8±0.2
Protein (Nx6.25)	12.3±0.4	11.1±0.3	12.2±0.3
Lipid	4.0±0.2	3.5±0.1	3.9±0.2
Ash	2.8±0.1	2.6±0.1	2.6±0.1
Carbohydrate[b]	68.4	70.6	69.9
Soluble fibre[c]	0.9	0.8	0.0
Insoluble fibre[c]	4.6	4.8	4.6
Total fibre[c]	5.5	5.6	4.6
Energy (kcal)	359	359	364

[a] Values are means±SD ($n=3$).
[b] Values obtained by difference.
[c] Values are means ($n=4$).

Table 5
Effects of control (AIN93M) and hypercholesterolemic diets on weight gain. food intake, feacal volum, serum cholesterol and triacylgycerol concentrations in rats at the 1st Step (1st–26th day)

Group[a]	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Food intake (g)	Fecal volume (g)	Serum cholesterol (mmol/l)	Serum triacylgycerol (mmol/l)
CONT	200.02±13.07	241.75±11.87	41.73±5.59a	484.86±26.37a	26.40±2.07a	1.00±0.24a	3.14±0.42a
HYPER[1]	200.14±45.94	249.04±55.03	48.90±9.79ab	371.29±7.99b	15.37±1.87b	4.99±0.57b	4.45±0.56b
HYPER[2]	193.03±9.49	255.48±14.53	62.44±14.15b	363.65±34.93b	19.24±3.29b	5.41±1.19b	4.45±0.52b
HYPER[3]	194.39±6.99	262.63±20.35	68.25±17.86b	376.65±54.67b	18.84±4.25b	5.23±1.27b	4.50±0.61b
HYPER[4]	201.04±8.05	269.64±5.19	68.60±11.07b	389.58±26.38b	19.46±3.11b	5.40±0.52b	4.42±0.64b
HYPER[5]	207.34±13.07	275.95±15.09	68.61±11.81b	383.00±67.58b	19.23±2.86b	5.02±0.93b	4.51±0.55b

[a] Values are means±SD ($n=6$). Values in a column with different letters are significantly different ($P<0.05$).

Table 6
Effects of experimental diets on weight gain, food intake, fecal volume, serum cholesterol and triacylglycerol concentrations and relative liver weight in rats at the 2nd Step (26th–52nd day)

Group[a]	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Food intake (g)	Feacal volume (g)	Serum cholesterol (mmol/l)	Serum triacylglycerol (mmol/l)	Relative liver weight (%)
CONT	241.75±15.33	340.05±26.80	98.30±12.53a	452.45±34.98a	32.06±2.36a	1.71±0.13a	4.83±0.28a	2.90±0.24a
HYPER	249.04±71.04	321.15±60.38	72.11±13.80b	368.98±82.74b	14.81±3.49b	7.78±1.45b	4.32±0.83a	5.85±0.38b
GUAR	255.48±14.53	327.04±11.75	71.57±8.11b	489.06±18.85ac	32.26±3.80a	1.43±0.12a	1.43±0.07b	3.21±0.35a
XANT	262.63±20.35	340.98±33.21	78.35±14.04b	528.50±34.36c	51.85±4.69c	1.41±0.24a	2.30±0.21c	3.27±0.32a
PGCS	269.64±5.19	333.57±12.35	63.93±10.97b	503.27±19.69ac	42.21±2.53d	1.54±0.14a	1.78±0.26bc	3.53±0.20a
CELL[b]	275.95±15.09	349.49±11.50	73.54±8.28b	515.61±13.84c	39.67±1.05d	1.42±0.11a	1.82±0.16bc	3.66±0.54a

[a] Values are means±SD ($n=6$). Values in a column with different letters are significantly different ($P<0.05$).
[b] This group CELL was used in order to compare the lipids serum alterations promoved by guar gum, xanthan gum and pre-gelatinized starch supllementation in the experimental diets.

14.8%). Practically the same fecal volume was observed between the CONT and GUAR groups and between the PGCS and CELL groups. The serum total cholesterol concentrations reached at the end of the second phase demonstrated significant alterations only in terms of a reduced dietary lipid content and altered level of saturation, since the cholesterol concentration was significantly higher in the HYPER group (7.8 mmol/l) than in the other groups (1.5 mmol/l). However, triacylglycerol concentrations were significantly altered, with the XANT group showing a significantly higher value (2.3 mmol/l) than the CELL group (1.8 mmol/l), while the GUAR and PGCS groups did not differ significantly from the latter (1.4 and 1.8 mmol/l, respectively). Similarly, a difference in relative liver weight was observed only for the HYPER group (5.8%), which showed a 77% higher weight than the mean obtained for the other groups (3.3%). These values are in agreement with the 5.3 and 3.4% values reported by Zulet et al. (1999) for hypercholesterolemic and control groups, respectively, after 26 days of experimentation. Finally, the CONT group showed an increase in cholesterol from 1.0 to 1.7 mmol/l and in triacylglycerol from 3.1 to 4.8 mmol/l during the two phases of the experiment, without any interference except for continuous weight gain of the animals. No significant difference in serum total cholesterol was observed between the CELL (1.4 mmol/l) and CONT groups (1.7 mmol/l) during the second phase, demonstrating that the alterations in dietary lipids were sufficient for this parameter to return to previous levels. However, the same effect could not be observed for the triacylglycerol concentration, with a significant reduction in triacylglycerol levels being observed when the composition of the diet was changed from hypertriglycemic, containing 25% saturated fat, to a more equilibrated formulation containing 4% unsaturated lipids.

4. Discussion

The capacity of polysaccharides such as guar gum, xanthan gum and pre-gelatinized corn starch to elevate the viscosity of solutions at low temperatures permits their use as thickeners in instant food formulations (Alves, Grossmann, & Silva, 1999). Mixtures of xanthan and guar form stable solutions with very high viscosity (Casas, Modhedano, & García-Ochoa, 2000). As shown in Table 3, this "functional" property seems to be independent of the soluble fibre content. Only a fraction of the hydrocolloids consists of soluble fibres that are able to form a highly viscous medium, which could alter lipid emulsification and lipolysis (Pasquier et al., 1996). As

shown in Tables 3, 4 and 5, the daily intake of the animals was 0.4 g hydrocolloid/100 kcal, corresponding to about 10 g/day for the human diet based on a daily supply of 2500 kcal. Similarly, soluble fibre intake was of the order of 0.26, 0.19 and 0.00 g/100 kcal day^{-1} for guar gum, xanthan gum and pre-gelatinized corn starch, respectively, corresponding to 6.5, 4.7 and 0.0 g/day for the human diet. These supplementation levels are compatible with those used in 67 experimental studies (2–10 g/day) selected and discussed in a meta-analysis aimed at quantifying the hypocholesterolemic effect of different dietary fibres (Brown, Rosner, Willet, & Sacks, 1999). On the other hand, diet supplementation with soluble fibres above these levels may impair the sensory quality of these formulations and may lead to disequilibrium in the diet as a whole and gastrointestinal discomfort (Hosobuchi et al., 1999; Yamamoto et al., 2000).

According to Levrat-Verny et al. (2000), a low percentage of hydrocolloid (1–2%) is sufficient to lower plasma cholesterol in rats fed experimental diets containing a moderate level of cholesterol (0.2%), because rats are rather unresponsive to low levels of hydrocolloids when fed cholesterol-free diets and, in this case, cholesterolemia is not significantly altered. However, food supplementation with hydrocolloids, as a process to render the diet functional or nutraceutic, should be accompanied by a recommendation regarding the minimum possible intake of cholesterol, saturated fat and trans fatty acids from the diet. The purpose of ingesting functional foods is to obtain an additional beneficial effect in relation to the previously adopted dietary interventions, thus contributing to a future reduction of the use of medications, to a more prolonged equilibrium of these biochemical parameters, and to an improved quality of life as a whole. Chronic consumption of functional foods does not exclude the need for traditional therapies based on the use of medications, the practice of physical exercise, the elimination of cigarette smoking and the maintenance of low-fat diet. For this reason, in the present study cholesterol-free diets with a low lipid content were chosen for supplementation.

Some hydrocolloids have been shown to be particularly effective as a cholesterol-lowering agent. They have the capacity to form gels in the small intestine which may trap organic material such as bile acids or sterols (Todd, Benfield, & Goa, 1990). Studies have shown that the intake of these hydrocolloids leads to a rapid increase in the viscosity of the gastrointestinal content and slow gastric emptying, providing a feeling of fullness that prevents overeating and, consequently, a lower intake of other nutrients (Ebihara & Schneeman, 1989). It should be noted that, in humans, the sensation of satiety can be induced by different factors, such as calorie content, composition and physical state of the diet, hormone concentration, and psychological factors, among others (Green, Wales, Lawton & Blundell, 2000; Hosobuchi et al., 1999; Pasquier et al., 1996; Peracchi, Santangelo, Conte, Fraquelli, Tagliabue, Gebbia & Porrini, 2000; Yeomans, Lartamo, Procter, Lee & Gray, 2001). This hypothesis has been extensively explored in the treatment of obesity by adding hydrocolloids to the formulation of slimming diets.

Another consequence of the elevated viscosity of the gastrointestinal content due to the higher intake of some hydrocolloids, is the reduced access of digestive enzymes caused by the complexing of nutrients within the polysaccharide matrix, which mainly consists of soluble fibres, as well as the complex formation with bile acids, thus removing cholesterol and, consequently, inducing a higher hepatic uptake of plasma cholesterol in order to maintain steady-state enterohepatic cycling. In this case, a greater proportion of bile acids would escape ileal reabsorption and then reach the large bowel, where hydrocolloids could impair this passive reabsorption by insolubilization (Favier, Bost, & Demigné, 1998; Levrat-Verny et al., 2000). An increase in intestinal excretion of steroids and their concomitant decrease in plasma was observed by Overton et al. (1994) in rats receiving a 10% guar gum-supplemented diet. In addition, these authors obtained a 27% reduction in serum cholesterol and found an increase in cholesterol-7-alpha-hydroxylase activity, an enzyme that catalyzes the oxidation of hepatic cholesterol to bile acids, and may increase cholesterol uptake from plasma in order to maintain steady-state enterohepatic bile acid cycling. Anderson et al. (1994) observed a significant reduction in serum cholesterol concentration in rats supplemented with 6% guar gum as soluble dietary fibre, while Favier et al. (1998) obtained a 25% reduction with 2.5% supplementation. Levrat-Verny et al. (2000) concluded that 1% hydrocolloids, such as guar and xanthan gums, in the diet, significantly reduce cholesterol in rats, due to the capacity of these compounds to accelerate the excretion of neutral steroids and to reduce the intestinal absorption of cholesterol. Similarly, other studies have reported a hypocholesterolemic effect as the result of the consumption of different types of starch, natural or modified, and attributed this property to the digestibility, composition (amylose/amylopectin ratio) and capacity of these compounds to elevate the viscosity in the lumen of the small intestine, which results in decreased intraluminal mixing (Favier et al., 1998; Hirao, Igarashi, Fukuda & Endo, 2000; Pasquier et al, 1996). However, the results shown in Table 6 suggest that, under the experimental conditions of the present study on rats, supplementation with 1.5% hydrocolloids was not sufficient to produce a significant hypocholesterolemic effect. These results agree with data reported by Trautwein, Kunath-Rau, and Erbersdobler (1998), suggesting that the capacity of the three different types of hydrocolloids evaluated in the present study (guar gum, xanthan gum and pre-gelatinized corn starch) to elevate the viscosity

of the gastrointestinal content is not a good predictor of their cholesterol-lowering potential, and that viscosity is apparently not a major factor involved in the hypolipidemic effect of these substances.

The mechanisms by which soluble fibres might interfere with triacylglycerol hydrolysis have been discussed but there has not yet been a definitive conclusion. In the present study, serum triacylglycerol concentration was reduced in all experimental groups compared to the CONT and HYPER groups, suggesting that the rat model used here was not adequate, since intake of the saturated diet during the second phase did not result in significant alterations in these parameters compared to the control diet. Comparing the cholesterol and triacylglycerol levels between the two experimental phases, only cholesterol concentration increased, while triacylglycerol levels remained constant, except for the diets containing hydrocolloids. Yamamoto et al. (2000) observed a hypotriglycerolemic effect in diabetic rats supplemented with different mixtures of 3% guar and xanthan gum and suggested that this effect was due to a delayed absorption of triacylglycerols in the small intestine caused by the high viscosity of the intestinal content. Pasquier et al. (1996) hypothesized that soluble fibres could alter emulsification and lipolysis of dietary triacylglycerol in the digestive tract, with possible involvement of viscosity-mediated mechanisms. However, the fact that serum triacylglycerol concentration was also reduced in the CELL group rules out this hypothesis as an explanation for the results obtained in the present study. Malkki et al. (1993) also did not observe significant differences in serum triacylglycerol concentration between rats submitted to a hypercholesterolemic diet (10 g/kg cholesterol and 2 g/kg cholic acid) and those receiving a normal maintenance diet after 3 weeks of supplementation. The authors emphasized the great difficulty in evaluating fibre preparations in animals due to the lack of a species with a lipid metabolism similar to that of humans and suggested the use of young animals in which lipid parameters better represent fibre-supplementation effects upon diet-induced hypertriacylglycerollmia than do adult animals. Hamsters could be a better option but they can break these fibres in the upper digestive tract, which disturbs their specific physico-chemical properties and influences their effects on cholesterol metabolism (Trautwein et al., 1998). According to Brown et al. (1999), different soluble fibres, including guar gum, did not significantly affect triacylglycerol concentration. Zulet et al. (1999) also did not observe differences in serum triacylglycerol concentration between rats submitted to a hypercholesterolemic diet and those submitted to a control diet. Lipids are digested by lipases, absorbed by enterocytes and released into plasma in the form of chylomicrons, which are transported to the liver and then to the different tissues for oxidation or storage (Dietschy, 1998). This physiological system is regulated by various factors that are difficult to control experimentally and that may have interfered with the results shown in Table 6. In comparison, the higher serum TG observed in the XANT group suggested that the viscosities of these three hydrocolloids should not be considered a good indication of their functional properties, as reported by Trautwein et al. (1998). Analysis of the relative liver weight revealed a significant effect of the hypercholesterolemic diet on lipid metabolism and an effect of the diet changes on morphological recovery of the hepatic tissue, suggesting that the time of supplementation used during the second phase was sufficient to reestablish the physiological steady-state of the animals with on the diet changes adopted. The last analysis also confirmed the low significance of the consumption of these hydrocolloids on lipid metabolism when compared to the effects of quantitative and qualitative changes in dietary lipids. Therefore, more attention should be paid to soluble fibres and other polysaccharides used to develop functional foods.

5. Conclusion

The addition of 1.5% hydrocolloids, such as guar gum, xanthan gum and pre-gelatinized cornstarch, to a cholesterol-free diet containing only 4% (mainly unsaturated) fatty acids, did not show any significant hypolipidemic effect, as demonstrated by the measurement of serum cholesterol and triacylglycerol concentrations in hypercholesterolemic rats. Hydrocolloids with similar functional properties, e.g. capacity to elevate the viscosity of solutions at low temperatures, do not present the same nutraceutical effect when applied in foods. Therefore, studies using concentrations compatible with human intake should be carried out before the indiscriminate use of hydrocolloids as ingredients in the development of functional or nutraceutical foods.

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