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Comparative effect of amidated pectin and psyllium on cholesterol homeostasis in rats

Communication

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Abstract: The effects of amidated pectin and psyllium on serum, hepatic and faecal cholesterol concentration were compared in female rats fed diets supplemented with palm fat and cholesterol at 50 and 10 g/kg, respectively. Control rats were fed a diet supplemented with cellulose at 60 g/kg. In treated rats, cellulose was replaced with either amidated pectin or psyllium. Amidated pectin and psyllium intake significantly decreased serum cholesterol from 3.41 μmol/ml (control) to 1.68 and 2.04 μmol/ml, respectively, and hepatic cholesterol from 31.9 μmol/g (control) to 7.2 and 9.0 μmol/g, respectively. Histology with lipid-staining Sudan Black B revealed that liver tissue from control rats was infiltrated with lipids, but staining was absent in livers of treated rats. No hepatic pathophysiology was apparent in treated rats. Amidated pectin and psyllium intake significantly increased faecal fat content. Faecal cholesterol content was significantly increased in rats that were fed amidated pectin, and non-significantly increased in rats that were fed psyllium. Body weight and food intake did not differ among treatment groups. In conclusion, amidated pectin, a novel sequestrant of sterols, demonstrated a similar effect on rat serum and hepatic cholesterol concentration to psyllium, which is a well-established hypocholesterolaemic agent.

Keywords: *Cholesterol • Amidated pectin • Psyllium • Rat*

1. Introduction

The association between plasma cholesterol concentration and coronary heart disease is well established; thus, the possibility of lowering plasma cholesterol concentration by interfering with the intestinal absorption of bile acids and cholesterol has been extensively investigated. Lowered uptake of bile acids from the intestine is compensated for by increased hepatic bile acid formation from its cholesterol precursor. As a portion of this precursor cholesterol comes from plasma, the result from increased bile acid formation is decreased cholesterolaemia. Various investigators have reported greater bile acid excretion *via* cholestyramine, a synthetic bile acid binding resin that is widely used to treat hypercholesterolaemia [1], and by psyllium, a hydrophilic, gel-forming fibre that is effective against functional disorders of the large bowel [2]. Several authors also tested *in vitro* adsorption of bile acids by aminated or amidated polysaccharides, including diethylaminoethyl cellulose [3], aminated dextran, pullan and cellulose [4], diethylaminoethyl chitosan [5] and N-octadecylpectinamide [6]. In contrast, reports on the *in vivo* effects of aminated or amidated polysaccharides are sporadic. Zuin *et al.* [7] reported that diethylaminoethyl dextran decreased serum bile acid and total cholesterol concentrations in patients with biliary cirrhosis. Aminated dextran was as effective as

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Crude protein	211 g
Crude fibre	99 g
Fat	90 g
Ash	66 g
L-lysine	14 g
D, L-methionine	4.8 g
Vitamin A	8.4 mg
Vitamin D_3	55 μg
Vitamin E	100 mg

Table 1. Chemical composition of the basal rat diet[a] (per kg).

[a] *Basal diet ingredients were soybean meal (extracted), fish meal, wheat, maize, oat, wheat meal, wheat bran, cellulose (60 g kg^{-1}), protected palm fat (50 g kg^{-1}), cholesterol (10 g kg^{-1}), limestone, dicalcium phosphate, salt and supplements of vitamins, trace elements and amino acids. Experimental diets were prepared by replacing cellulose with amidated pectin or psyllium.*

cholestyramine but was better tolerated. Marounek *et al.* [8] investigated the effects of amidated pectin on cholesterol homeostasis in rats. Amidated pectin significantly decreased the serum and hepatic concentrations of cholesterol and increased its concentration in faeces. Using female rats, we test a hypothesis that the lipid-lowering effects of amidated pectin are comparable to those of psyllium, which is a commonly used hypocholesterolaemic agent. Rats have the ability to convert excess dietary cholesterol to bile acids and, as such, are rather resistant to hypercholesterolaemic diets [9]. Female rats were chosen because female rats are more susceptible to dietary cholesterol [10].

2. Experimental Procedures

2.1 Material

The amidated pectin employed was N-octadecylpectinamide with a degree of substitution of 37%, prepared by heterogeneous amino-dealkoxylation of highly methoxylated citrus pectin [11]. Microcrystalline cellulose and cholesterol were supplied by Sigma-Aldrich (Prague, Czech Republic), psyllium was provided by Dr. Popov Ltd. (Planá, Czech Republic), and the protected palm fat was supplied by Agro-Best Ltd. (Běstovice, Czech Republic).

2.2 Animals and diets

Eighteen female Wistar rats (approximately 6 weeks of age) were housed individually in a temperature and humidity-controlled room. The animal facility was maintained on a 12 h light: 12 h dark daily photoperiod cycle, at a temperature of 22±1°C and a relative humidity of 60±5%. The rats were fed a basal rat diet ST-1 (Velaz Ltd., Lysolaje, Czech Republic) supplemented with microcrystalline cellulose, protected palm fat and cholesterol at concentrations of 60, 50 and 10 g kg^{-1}, respectively (Table 1). After one week, the rats were randomly divided into three groups of six animals each. Experimental diets were prepared by replacing the cellulose with either N-octadecylpectinamide or psyllium. Food and water were available *ad libitum*, and the rats were fed for four weeks. The experiment was approved by the Ethical Committee of the Institute of Animal Science.

2.3 Sampling

Faeces were collected during the last week of the experiment, pooled and stored at –40°C until further analysis. After four weeks of the diet, the rats were anesthetised by inhalation of Isofluran (Nicholas Piuramal India Ltd, London) and sacrificed by decapitation. Samples of mixed blood were withdrawn at slaughter to obtain serum. Livers were excised, and one half of each liver was fixed in buffered neutral formaldehyde [12] for histochemical examination. The second half was frozen and maintained at –40°C until further analysis.

2.4 Analyses

Serum triacylglycerol and total cholesterol concentrations were determined by using commercially available reagent kits supplied by BioVendor, Inc. (Brno, Czech Republic). The total lipids were extracted from livers and faeces with 2:1 chloroform-methanol [13]. Lipids in the extracts were determined gravimetrically after washing with 1% NaCl and 50% methanol. To determine hepatic cholesterol, lipids were saponified, and the unsaponified matter was extracted with diethyl ether [14]. Silyl derivates were prepared using TMCS and HMDS silylation reagents (Sigma-Aldrich, Prague, Czech Republic) and quantified on a gas chromatograph equipped with a SAC-5 capillary column (Supelco, Bellefonte, U.S.A.), which was operated isothermally at 285°C. The faecal cholesterol level was determined in freeze-dried samples by gas chromatography on a 25 m capillary column (CP-SIL 5CB, 0.25 mm i.d., Varian, U.S.A.).

2.5 Histology

Sections of liver tissue (10 µm thick) were cut using a freezing microtome Mikrotom M3 (Medexport,

	Control	Amidated Pectin	Psyllium
Initial weight (g)	160 ± 8	159 ± 6	156 ± 6
Final weight (g)	195 ± 8	197 ± 16	201 ± 16
Weight gain (g)	35 ± 4	38 ± 11	45 ± 13
Food intake (g d^{-1})	14.9 ± 0.8	14.9 ± 1.4	16.0 ± 1.2

Table 2. Effect of amidated pectin and psyllium on growth and food intake in female rats that were fed a cholesterol-containing diet. (Values are means and SD for six rats per diet).

	Control	Amidated Pectin	Psyllium
Serum concentrations			
Triacylglycerols (μmol ml^{-1})	0.81 ± 0.24	0.58 ± 0.14	0.89 ± 0.26
Total cholesterol (μmol ml^{-1})	3.41 ± 0.73^{a}	1.68 ± 0.22^{b}	2.04 ± 0.27^{b}
Hepatic concentrations			
Total lipids (mg g^{-1})	68.4 ± 7.0^{a}	49.9 ± 4.9^{b}	52.4 ± 5.1^{b}
Cholesterol (μmol g^{-1})	31.9 ± 7.5^{a}	7.2 ± 0.5^{b}	9.0 ± 0.6^{b}
Faecal concentration			
Dry matter (mg g^{-1})	634 ± 39^{a}	624 ± 46^{a}	407 ± 35^{b}
Total lipids (mg g DM^{-1})	155 ± 13^{a}	230 ± 18^{b}	312 ± 82^{c}
Cholesterol (μmol g DM^{-1})	34.9 ± 4.5^{a}	61.3 ± 8.2^{b}	42.7 ± 3.2^{a}

Table 3. Effect of amidated pectin and psyllium on the serum concentrations of triacylglycerols and cholesterol, the hepatic concentration of total lipids and cholesterol, and the faecal concentration of dry matter, faecal total lipids and faecal cholesterol in female rats that were fed a cholesterol-containing diet. (Values are means ±SD for six rats per diet).

^{a,b,c} *Values in the same row with different superscripts were significantly different ($P<0.05$)*

Moscow, Russia) and stained with diazo dye Sudan black B to reveal lipids. Standard haematoxylin-stained paraffin sections were prepared to detect possible pathomorphologic changes. All sections were stained in one batch to ensure comparability among the different groups. Sections were examined microscopically using a microscope equipped with an Olympus CAMEDIA C-5050 Zoom digital camera.

2.6 Statistics

Data were statistically analysed by one-way analysis of variance using the GLM procedure of SAS, version 8.2 [15]. In the case of a significant difference ($P<0.05$) groups were then compared by the Tukey's test. The results are expressed as means ± SD.

3. Results

Body weight and food intake during the four week study did not differ among treatment groups ($P>0.05$; Table 2). There was no statistically significant treatment effect on serum triacylglycerol (Table 3). In contrast, the total serum cholesterol level in rats fed amidated pectin and psyllium (1.68 and 2.04 µmol ml^{-1}, respectively) was significantly lower than in the control rats (3.41 µmol ml^{-1}). Amidated pectin and psyllium significantly decreased the hepatic fat concentration and reduced the cholesterol concentration to 22.6 and 28.3% of the control value (31.9 µmol g^{-1}), respectively. The hepatic cholesterol correlated significantly with total

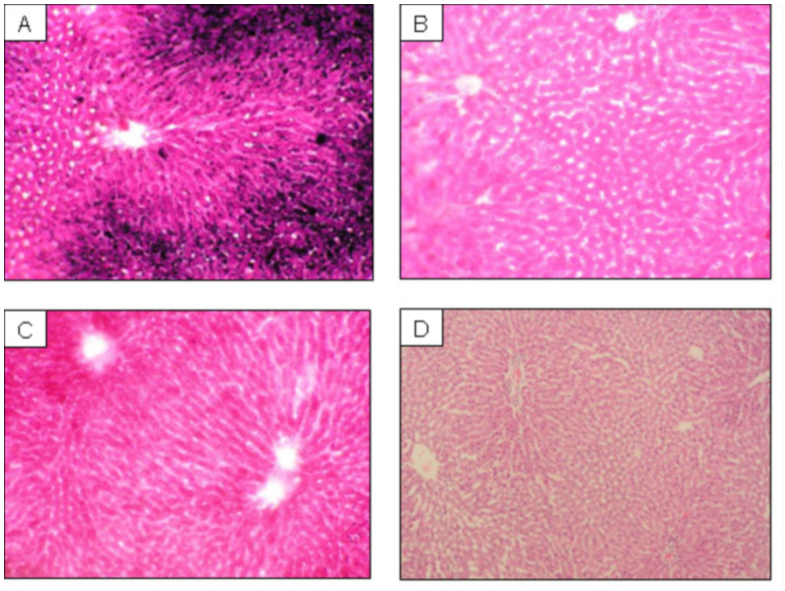

Figure 1. Histological analysis of liver tissue of rats that were fed a control diet (A) or a diet supplemented with amidated pectin (B,D) or psyllium (C). Sections of liver tissue were stained with Sudan black B dye (A,B,C) or haematoxylin (D). Magnification x 50. The liver tissue of the control rats was infiltrated with lipids that stain black. The Sudan black B-positive material was absent in the livers of treated rats.

serum cholesterol (r=0.92; P<0.001). The concentration of dry matter in the faeces of rats that were fed psyllium was significantly lower than in the faeces of other rats. Both amidated pectin and psyllium significantly increased the lipid concentration in faecal dry matter. The faecal cholesterol concentration was significantly increased in rats that were fed amidated pectin and non-significantly increased in rats that were fed psyllium.

Figures 1A, 1B and 1C present the appearance of hepatic tissue stained with the fat-soluble dye Sudan black B. Figure 1D presents the appearance of hepatic tissue stained with haematoxylin. Figure 1A shows that the hepatic tissue of the control rats was infiltrated with lipids that stain black with Sudan black B. The infiltrated fat was present mainly in the periportal zone of the liver. The Sudan black B-positive material was absent in the livers of treated rats. No pathophysiological changes were apparent in livers of amidated pectin-fed rats.

4. Discussion

Serum cholesterol concentration in rats that were fed the cholesterol-enriched control diet (3.41 μmol ml^{-1}) exceeded the median value of the cholesterol concentration reported for female Wistar rats (1.50 μmol ml^{-1}; [16]). In two previous experiments [8] with male rats fed an almost identical diet containing cholesterol at 10 g kg^{-1}, the serum cholesterol concentration was 1.99 and 2.08 μmol ml^{-1}. Furthermore, the effect of amidated pectin was less pronounced in male rats; only a moderate reduction in serum cholesterol was observed in treated animals [8]. The data of Terpstra *et al.* [10] are consistent with these findings. In the female rats, the feeding of casein-containing diets resulted in significantly higher levels of serum cholesterol than from soybean-protein–containing diets. However, no such differences were observed in male rats. The authors concluded that female rats were more susceptible to induced changes in serum cholesterol than male rats. Sex-related differences were also observed with induced hypercholesterolaemia in rats, as reported by Radzki *et al.* [17].

Psyllium is a hydrophilic, gel-forming fibre that disrupts the uptake of luminal cholesterol by the small intestine. In rats, psyllium increases the faecal excretion of total steroids and bile acids, presumably *via* a greater viscosity of intestinal contents [18]. Both amidated pectin and psyllium decreased the serum cholesterol to a similar extent. In addition, changes in hepatic cholesterol paralleled those in serum cholesterol. Hepatic cholesterol concentrations in rats fed different diets are quite variable, ranging from 2-3 μmol g^{-1} [19] to 90 μmol g^{-1} [20]. Cholesterol-lowering agents (amidated pectin and psyllium) significantly decreased the hepatic lipid concentration from 68.4 to 49.9 and 52.4 mg g^{-1}, apparently at the expense of infiltrated hepatic fat, which was absent in rats that were fed amidated pectin and psyllium. The effect of amidated pectin and psyllium on histological data was more pronounced than on the hepatic lipid concentration, probably due to different affinities of Sudan black B dye for infiltrated fat and structural lipids. The reduced serum and hepatic cholesterol levels in the rats receiving amidated pectin and psyllium was accompanied by increased faecal cholesterol. In conclusion, amidated pectin, a novel sequestrant of sterols, and psyllium, a well-described lipid-lowering agent, demonstrated similar effects on rat serum cholesterol and hepatic cholesterol content.

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