The influence of enzyme-resistant starch on cholesterol metabolism in rats fed on a conventional diet

K. Vanhoof and R. De Schrijver*

Laboratory of Nutrition, Catholic University of Leuven, Kard. Mercierlaan 92, 3001 Leuven, Belgium

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Male Wistar rats were fed on a conventional diet containing normal corn starch or 6 % enzyme-resistant starch originating from either raw or retrograded high-amylose corn starch. Furthermore, the diets were either cholesterol-free or contained 1 % cholesterol and 0·1 % cholic acid. The main objective of this study was to investigate whether the addition of enzyme-resistant starch to a rat conventional diet had any effect on cholesterol metabolism. Therefore, plasma and liver cholesterol concentrations, plasma HDL:LDL cholesterol ratios and neutral steroid and bile acid excretion were determined. No significant effect of enzyme-resistant starch feeding on plasma and liver cholesterol concentrations was found. However, consumption of raw or retrograded high-amylose corn starch resulted in a decrease in esterified and total liver cholesterol concentrations of 24 and 22 %, respectively. This was accompanied by a reduction in plasma esterified and total cholesterol levels of 4 % and a tendency to higher daily faecal coprostanol and total bile acid excretion.

Resistant starch: Cholesterol metabolism: Rats

A number of recent studies (de Deckere *et al*. 1993, 1995; Younes *et al*. 1995; Vanhoof & De Schrijver, 1997) show that enzyme-resistant starch (RS) might have a hypocholesterolaemic effect, both in normo- and hypercholesterolaemic rats. This effect may be caused by lower cholesterol absorption, increased neutral steroid and bile acid excretion or increased synthesis of fermentation products like propionic acid, which in turn may decrease cholesterol synthesis in the liver (Chen *et al*. 1984). Yet, this last factor is considered controversial as *in vivo* propionate concentrations might not be high enough to decrease the activity of hydroxy-methylglutaryl-CoA reductase (*EC* 1·1.1·88), the rate-limiting enzyme in hepatic cholesterol synthesis (Illman *et al*. 1988; Beaulieu & McBurney, 1992).

In this study the effect of RS on cholesterol metabolism in normo- as well as hypercholesterolaemic rats was investigated by measuring plasma cholesterol concentrations and faecal steroid excretion. High-amylose corn starch and retrograded high-amylose corn starch were used as sources of RS types 2 and 3 respectively. In contrast with the above mentioned studies which were performed with semi-synthetic diets, we wanted to test the effect of both RS sources when added to a conventional diet. Consequently, we could examine whether the hypocholesterolaemic effect of RS that was observed in our previous study with semi-synthetic diets (Vanhoof & De Schrijver, 1997) was influenced by diet composition.

Materials and methods

Enzyme-resistant starch sources

High-amylose corn starch (Hylon VII®) and retrograded high-amylose corn starch (Novelose®) were used as sources of RS types 2 and 3 respectively. Fibre sources were obtained from Unilever (Vlaardingen, The Netherlands) and National Starch and Chemical Company (Neustadt, Germany). Hylon VII® contained 55·4 % and Novelose® 41·1 % RS. Normal corn starch (Meritena A, Amylum, Belgium) was used as a reference.

Animals and diets

All diets contained the same amounts of barley, wheat, cassava and soyabean meal as the major ingredients (Table 1). Two normocholesterolaemic test diets were devised to contain 6 % RS type 2 or 3. Therefore, 10·8 % Hylon VII® (RS2–) or 14·6 % Novelose® (RS3–) were added at the expense of the normal corn starch in the normocholesterolaemic control diet (C–). Three other diets (C+, RS2+ and RS3+) were devised which were similar to the preceding ones, except that they contained 1 % cholesterol and 0·1 % cholic acid replacing diatomaceous earth. Cholic acid was added to enhance the hypercholesterolaemic effect of the cholesterol feeding.

The animal care procedures were conducted under

Abbreviation: RS, resistant starch.

***Corresponding author:** Professor R. De Schrijver, fax +32 16 321971, email Remi.DeSchrijver@agr.kuleuven.ac.be

Table 1. Composition of the diets (g/kg)

Ingredient	C–	RS2–	RS3–	C+	RS2+	RS3+
Barley	371	371	371	371	371	371
Wheat	173	173	173	173	173	173
Cassava	112	112	112	112	112	112
Soyabean meal	43	43	43	43	43	43
Corn starch	203	95	57	203	95	57
Hylon VII®*	–	108	–	–	108	–
Novelose®†	–	–	146	–	–	146
Cellulose	16	16	16	16	16	16
Sucrose	13	13	13	13	13	13
Corn oil	12·5	12·5	12·5	12·5	12·5	12·5
Vitamin, mineral and amino acid mix	45·8	45·8	45·8	45·8	45·8	45·8
Diatomaceous earth	11	11	11	–	–	–
Cholesterol	–	–	–	10	10	10
Cholic acid	–	–	–	1	1	1
Total dietary fibre‡	177	191	219	190	196	224

C, control diet without added resistant starch (RS); RS2, diet with 6 % RS type 2; RS3, diet with 6 % RS type 3; –, diet without added cholesterol and cholic acid; +, diet with 1 % cholesterol and 0·1 % cholic acid.
* Hylon VII®, high-amylose corn starch obtained from Unilever, Vlaardingen, The Netherlands and National Starch and Chemical Company, Neustadt, Germany.
† Novelose®, retrograded high-amylose corn starch obtained from Unilever, Vlaardingen, The Netherlands and National Starch and Chemical Company, Neustadt, Germany.
‡ Measured according to the procedure of Prosky & Lee (1992).

Table 2. Plasma cholesterol concentrations*
(Mean values with their standard errors for eight animals)

	Cholesterol concentration (mmol/l)			HDL:LDL cholesterol ratio
Diet	Esterified	Free	Total	
C–	1·11 (0·02)	0·48 (–0·32)	1·59 (0·19)	1·44 (0·14)
RS2–	1·08 (0·02)	0·46 (–0·34)	1·54 (0·18)	1·61 (0·20)
RS3–	1·07 (0·02)	0·48 (–0·32)	1·55 (0·19)	1·47 (0·16)
C+	2·39 (0·36)	0·53 (–0·29)	2·91 (0·45)	0·76 (–0·13)
RS2+	2·31 (0·34)	0·51 (–0·30)	2·82 (0·43)	0·79 (–0·12)
RS3+	2·27 (0·33)	0·48 (–0·31)	2·77 (0·42)	0·73 (–0·15)
SEM	0·09 (0·02)	0·01 (0·01)	0·10 (0·02)	0·04 (0·02)
Probability values for assessing specific comparisons between diet means (*P*)† :				
Cholesterol‡	0·0001	NS	0·0001	0·0001
RS‡	NS	NS	NS	NS
Cholesterol × RS‡	0·0001	NS	NS	NS

C, control diet without added resistant starch (RS); RS2, diet with 6 % RS type 2; RS3, diet with 6 % RS type 3; –, diet without added cholesterol and cholic acid; +, diet with 1 % cholesterol and 0·1 % cholic acid.
* Results are presented as means adjusted for initial body weight with standard errors of the means (SEM). Means of logarithms, adjusted for initial body weight, with their SEM are presented in parentheses. For details of diets and experimental procedures see Table 1 and pp. 193–194.
† Comparisons were performed in the logarithmic scale; no interaction implies similar proportional changes with RS at each level of cholesterol.
‡ Cholesterol, RS, main effect of cholesterol and enzyme-resistant starch addition on the measured variable; Cholesterol × RS, interaction between cholesterol and RS.

protocols of the Foundation for Scientific Research. Forty-eight male Wistar rats, weighing about 200 g, were individually housed in wire-bottomed metabolism cages in a well ventilated room with the dark period from 20.00 to 08.00 hours. Cages were positioned on a rack and placed in front of tube lighting. Rats were allocated to the cages per group in a vertical manner in order to exclude mistakes in food administration and influences of temperature and lighting. Deionized water and food were given *ad libitum*. At the beginning of the experiment, animals were divided into two groups of twenty-four rats each, both groups having the same initial mean body weight. During the first 2 weeks one group received the normocholesterolaemic control diet (C–), containing neither added RS, nor cholesterol and cholic acid. The second group received the hypercholesterolaemic control diet (C+). At the end of this period both groups were divided into three subgroups of eight rats each, all having approximately the same initial mean body weight. One subgroup continued to receive the control diet (C– and C+), while the other two groups were switched over to the test diets with 6 % RS type 2 (RS2– and RS2+) or type 3 (RS3– and RS3+). All diets were fed for another 5 weeks. During this period water and food intakes were recorded daily. Faeces were collected daily during the last 3 weeks of the experiment. Faecal collections from each rat were pooled, freeze-dried, ground and analysed for steroid content. At the end of the experiment, rats were anaesthetized with diethyl ether and killed by decapitation. The liver was removed, blotted and stored at –80° until analysis for lipid content. Blood was collected in heparinized tubes, plasma prepared by centrifugation and stored at –80° until analysis for cholesterol content.

Analytical methods

The total dietary fibre content of the diets was determined according to the procedure of Prosky & Lee (1992).

Plasma and liver lipids were extracted with chloroform–methanol (2:1, v/v) and determined by TLC in combination with flame ionization detection (Ackman *et al*. 1990; De Schrijver & Vermeulen, 1991). Free and esterified cholesterol, triacylglycerols and phospholipids were quantified after separation on chromarods (type-SIII) using petroleum ether–diethyl ether–formic acid (85:15:0·1, by vol.) as the solvent system. Plasma HDL- and LDL-cholesterol concentrations were measured enzymically with a Boehringer kit (Boehringer Pharma, Mannheim, Germany).

Individual faecal neutral steroids and bile acids were measured according to methods described by Miettinen *et al*. (1965) and by Grundy *et al*. (1965) respectively.

Total 3α-hydroxy-bile acids were determined after extraction of the samples with *t*-butanol–water (1:1, v/v). The extracted bile acids were quantified enzymically as described by Turley & Dietschy (1978).

Statistical analysis of data

Experimental data are presented as means adjusted for initial body weight with SEM. Data were subjected to a logarithmic conversion in order to transform the observations to a scale of constant variance. Therefore, means of logarithms adjusted for body weight with standard errors of the logarithmic means are also presented. As both normo- and hypercholesterolaemic diets were fed, data were subjected to a two-way ANOVA using the procedure of SAS (Statistical Analysis Systems, 1988) with cholesterol and RS consumption as main effects. The cholesterol × RS interaction was also tested. Initial body weight was also

Table 3. Liver cholesterol concentrations*
(Mean values with their standard errors for eight animals)

Diet	Cholesterol concentration (μmol/g)		
	Esterified	Free	Total
C–	1·80 (0·29)	4·22 (0·62)	6·01 (0·79)
RS2–	1·78 (0·29)	4·29 (0·63)	6·10 (0·79)
RS3–	1·90 (0·31)	4·11 (0·61)	6·00 (0·79)
C+	75·30 (1·85)	6·56 (0·81)	81·85 (1·89)
RS2+	55·64 (1·68)	6·64 (0·82)	62·28 (1·74)
RS3+	58·61 (1·64)	6·47 (0·81)	65·07 (1·72)
SEM	3·44 (0·14)	0·12 (0·01)	3·51 (0·03)
Probability values for assessing specific comparisons between diet means (*P*)† :			
Cholesterol‡	0·0001	0·0001	0·0001
RS‡	NS	NS	NS
Cholesterol × RS‡	0·0001	NS	NS

C, control diet without added resistant starch (RS); RS2, diet with 6 % RS type 2; RS3, diet with 6 % RS type 3; –, diet without added cholesterol and cholic acid; +, diet with 1 % cholesterol and 0·1 % cholic acid.
* Results are presented as means adjusted for initial body weight with standard errors of the means (SEM). Means of logarithms, adjusted for initial body weight, with their SEM are presented in parentheses. For details of diets and experimental procedures, see Table 1 and pp. 193–194.
† Comparisons were performed in the logarithmic scale; no interaction implies similar proportional changes with RS at each level of cholesterol.
‡ Cholesterol, RS, main effect of cholesterol and enzyme-resistant starch on the measured variable; Cholesterol × RS, interaction between cholesterol and RS.

Table 4. Faecal excretion of neutral steroids*
(Mean values with their standard errors for eight animals)

Diet	Faecal excretion (μmol/day)	
	Coprostanol	Cholesterol
C–	6·43 (0·81)	11·15 (0·99)
RS2–	6·68 (0·83)	10·72 (0·97)
RS3–	8·13 (0·91)	9·87 (0·97)
C+	31·53 (1·49)	278·64 (2·44)
RS2+	37·50 (1·56)	270·24 (2·42)
RS3+	44·11 (1·60)	254·51 (2·40)
SEM	1·38 (0·02)	3·10 (0·01)
Probability values for assessing specific comparisons between diet means (*P*)† :		
Cholesterol‡	0·0001	0·0001
RS‡	NS	NS
Cholesterol × RS‡	0·0001	NS

C, control diet without added resistant starch (RS); RS2, diet with 6 % RS type 2; RS3, diet with 6 % RS type 3; –, diet without added cholesterol and cholic acid; +, diet with 1 % cholesterol and 0·1 % cholic acid.
* Results are presented as means adjusted for initial body weight with standard errors of the means (SEM). Means of logarithms, adjusted for initial body weight, with their SEM are presented in parentheses. For details of diets and experimental procedures, see Table 1 and pp. 193–194.
† Comparisons were performed in the logarithmic scale; no interaction implies similar proportional changes with RS at each level of cholesterol.
‡ Cholesterol, RS, main effect of cholesterol and enzyme-resistant starch on the measured variable; Cholesterol × RS, interaction between cholesterol and RS.

included in the model as a covariant to adjust to a common initial weight. Values were considered significantly different at the 0·05 level. Statistical comparison between results obtained in this study and the study reported previously (Vanhoof & De Schrijver, 1997) was done after logarithmic conversion of the data. Data were subjected to three-way ANOVA using SAS, with type of diet, cholesterol and RS as main effects. In addition, the interactions between type of diet and cholesterol, type of diet and RS and between cholesterol and RS were also tested.

Results

Water intake, food intake and growth performance

There were no significant differences for food and water intakes and growth rate with different diet regimens. Mean daily food and water intakes were 20·0 g/d (SE 0·2) and 17·8 ml/d (SE 0·4) respectively. The mean growth rate was 1·65 g/d (SE 0·05).

Plasma cholesterol concentrations

There was no significant influence of RS consumption on free, esterified, and total cholesterol, nor was there any influence on HDL:LDL cholesterol ratios (Table 2). Addition of cholesterol and cholic acid to the test diets resulted in significantly higher concentrations of esterified, free and total cholesterol and in significantly lower HDL:LDL cholesterol ratios compared with the normocholesterolaemic animals. No significant interaction between RS and cholesterol supplementation on plasma cholesterol concentrations was found, indicating similar proportional changes due to RS consumption in normo- as well as hypercholesterolaemic rats.

Liver cholesterol concentrations

Table 3 shows the results of the effect of feeding RS on liver esterified, free and total cholesterol concentrations in normo- and hypercholesterolaemic rats. Feeding RS had no significant effect on the various cholesterol levels. However, consumption of RS types 2 and 3 resulted in a biologically important decrease in esterified and total cholesterol of 24 and 22 % respectively. This was accompanied by a reduction in plasma esterified and total cholesterol of 4 %. Conversely, addition of cholesterol and cholic acid to the diets resulted in significantly higher liver cholesterol values. No significant interaction between RS and cholesterol supplementation on liver cholesterol concentrations was found.

Faecal neutral steroid excretion

Data on the influence of dietary supplementation of cholesterol and RS on faecal coprostanol and cholesterol excretion are presented in Table 4. No significant interaction between RS and cholesterol supplementation on faecal neutral steroid excretion was found. In addition, no statistically significant effect of RS consumption on cholesterol excretion was observed. However, RS consumption tended ($P = 0·07$) to cause an increase in faecal coprostanol excretion. Supplementation of the diet with RS type 3 caused a biologically important rise in coprostanol output of 25 and 40 % in normo- and hypercholesterolaemic rats respectively. Faecal cholesterol output decreased by 4 and 9 % under the same circumstances. RS type 2 increased the

Table 5. Faecal bile acid excretion (μmol/day)*
(Mean values with their standard errors for eight animals)

	C–	RS2–	RS3–	C+	RS2+	RS3+	SEM
LCA	4·74 (0·66)	5·50 (0·73)	6·78 (0·82)	14·35 (1·13)	16·50 (1·20)	18·50 (1·26)	0·46 (0·02)
DCA	3·90 (0·56)	4·07 (0·58)	4·32 (0·59)	17·80 (1·22)	20·30 (1·29)	20·04 (1·30)	0·48 (0·02)
CDCA	0·65 (–0·24)	0·78 (–0·14)	0·82 (–0·11)	1·50 (0·12)	1·71 (0·22)	3·23 (0·46)	0·13 (0·02)
CA+α-MCA	1·28 (0·01)	1·37 (0·11)	1·54 (0·15)	7·38 (0·83)	7·66 (0·84)	8·40 (0·88)	0·45 (0·03)
HDCA	5·20 (0·69)	6·33 (0·78)	6·74 (0·76)	7·26 (0·73)	13·71 (1·04)	11·70 (0·94)	0·87 (0·04)
UDCA	1·65 (0·20)	2·09 (0·30)	2·13 (0·32)	3·03 (0·44)	3·99 (0·58)	3·73 (0·56)	0·12 (0·02)
KLCA	2·48 (0·38)	1·87 (0·23)	2·35 (0·31)	8·14 (0·89)	7·59 (0·87)	7·79 (0·89)	0·22 (0·02)
HCA	4·74 (0·67)	5·09 (0·69)	5·32 (0·70)	4·60 (0·61)	5·85 (0·76)	5·93 (0·75)	0·24 (0·02)
β-MCA	1·03 (–0·05)	0·97 (–0·07)	0·82 (–0·14)	4·15 (0·56)	4·62 (0·64)	4·06 (0·58)	0·23 (0·03)
ω-MCA	2·13 (0·23)	2·35 (0·25)	2·79 (0·35)	13·00 (1·03)	14·16 (1·10)	16·92 (1·21)	0·75 (0·04)
Total	22·04 (1·33)	23·73 (1·36)	26·67 (1·40)	76·64 (1·86)	90·30 (1·94)	93·34 (1·97)	2·09 (0·02)
Total (enz.)	26·11 (1·40)	31·74 (1·49)	32·36 (1·50)	106·48 (2·02)	109·17 (2·03)	100·92 (2·00)	1·09 (0·01)
Primary:secondary	0·35 (–0·47)	0·34 (–0·49)	0·27 (–0·67)	0·28 (–0·56)	0·26 (–0·59)	0·27 (–0·59)	0·01 (0·03)

LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; CA, cholic acid; α-MCA, α-muricholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid; KLCA, 7- and 12-ketolithocholic acids; HCA, hyocholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid; Total, sum of all individual bile acids mentioned; Total (enz.), 3α-hydroxy-bile acids quantified as described on p. 194; Primary:secondary, (CA+α-MCA+β-MCA+HCA+CDCA):(LCA+DCA+HDCA+UDCA+KLCA+ω-MCA); C, control diet without added resistant starch (RS); RS2, diet with 6 % RS type 2; RS3, diet with 6 % RS type 3; –, diet without added cholesterol and cholic acid; +, diet with 1 % cholesterol and 0·1 % cholic acid.
* Results are presented as means adjusted for initial body weight with standard errors of the means (SEM). Means of logarithms, adjusted for initial body weight, with their SEM are presented in parentheses. For details of diets and experimental procedures, see Table 1 and pp. 193–194.

Table 6. The probability values from ANOVA for assessing specific comparisons of faecal bile acid excretions*†

Bile acid	Cholesterol‡	RS‡	Cholesterol × RS‡
LCA	0·0001	0·0122	NS
DCA	0·0001	NS	NS
CDCA	0·0001	NS	NS
CA+α-MCA	0·0001	NS	NS
HDCA	NS	NS	NS
UDCA	0·0001	0·0365	NS
KLCA	0·0001	NS	NS
HCA	NS	NS	NS
β-MCA	0·0001	NS	NS
ω-MCA	0·0001	NS	NS
Total	0·0001	NS	NS
Total (enz.)	0·0001	0·0693	0·0290
Primary:secondary	NS	NS	NS

LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; CA, cholic acid; α-MCA, α-muricholic acid; HDCA, hyodeoxycholic acid; UDCA, ursodeoxycholic acid; KLCA, 7- and 12-ketolithocholic acids; HCA, hyocholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid; Total, sum of all individual bile acids mentioned; Total (enz.), 3α-hydroxy-bile acids quantified as described on p. 194; Primary:secondary, (CA+α-MCA+β-MCA+HCA+CDCA):(LCA+DCA+HDCA+UDCA+KLCA+ω-MCA).
* For details of diets and experimental procedures, see Table 1 and pp. 193–194.
† Comparisons were performed in the logarithmic scale; no interaction implies similar proportional changes with RS at each level of cholesterol.
‡ Cholesterol, RS, main effect of cholesterol and enzyme-resistant starch addition on the measured variable; Cholesterol, × RS, interaction between cholesterol and RS.

coprostanol and decreased the cholesterol excretion by 4 % in normocholesterolaemic rats and with 19 and 3 % in hypercholesterolaemic rats respectively. Hypercholesterolaemic rats had significantly higher faecal coprostanol and cholesterol excretion in comparison with rats receiving the normocholesterolaemic test diets.

Faecal bile acid excretion

Primary bile acid excretion was calculated as the sum of cholic acid, chenodeoxycholic acid, hyocholic acid and α- and β-muricholic acids (Eyssen & Robben, 1989). The secondary bile acids are ω-muricholic acids, lithocholic acid, deoxycholic acid, hyodeoxycholic acid, ursodeoxycholic acid and 7- and 12-ketolithocholic acids.

Daily excretion of lithocholic acid and ursodeoxycholic acid was significantly higher when RS was fed (Tables 5 and 6). This resulted in a tendency ($P = 0{\cdot}07$) towards a higher excretion of total bile acids. However, this increase was only found when total 3α-hydroxy-bile acid excretion was determined enzymically.

Hypercholesterolaemic rats had higher faecal excretion of all individual bile acids, except hyocholic and hyodeoxycholic acids, as well as total bile acids compared with normocholesterolaemic groups. No effect of cholesterol and RS feeding on the primary:secondary bile acid ratio was observed.

Discussion

Addition of 6 % RS in combination with cholesterol and cholic acid to a conventional rat diet had no effect on daily food and water intakes or on daily growth. These observations agree with results obtained by Gee *et al*. (1991), de Deckere *et al.* (1993, 1995) and Schulz *et al*. (1993). Conversely, addition of 10 % RS originating from high-amylose corn starch or 20 % resistant potato starch resulted in an increase in food intake of 14 and 36 % respectively (Mallett *et al*. 1988). This might be due to the fact that the animals adapted to the lower energy content of the test diets. Also, higher growth values were observed in rats fed on a diet containing 10 % retrograded corn starch (Faulks *et al*. 1989). Although semi-synthetic diets were used in all studies reported, the effect of RS on food intake and growth might depend on the source and dose of RS added to the diet. This might explain why rather different results were found.

Replacing highly digestible starch in the diet by RS can lower serum cholesterol concentrations in normo- as well as hypercholesterolaemic rats (de Deckere *et al*. 1993, 1995; Younes *et al*. 1995; Vanhoof & De Schrijver, 1997). Dietary fibre in general, and consequently RS in particular, might influence the cholesterol metabolism through one or more of

the following mechanisms: increased faecal bile acid excretion; altered intestinal absorption, metabolism and release of fat and cholesterol; reduced insulin-stimulated cholesterol synthesis; and the systemic effects of fermentation by-products like propionic acid and their effects on hepatic cholesterol synthesis. It has been shown that RS has the capacity to form inclusion complexes with bile acids due to its helical structure (Abadie *et al*. 1994). However, not every type of RS can bind bile acids. It is not expected that RS in the shape of granules can bind bile acids. The fact that RS consumption influences bile acid solubility was indicated by the studies of van Munster *et al*. (1993, 1994) showing a decrease of soluble deoxycholic, lithocholic and chenodeoxycholic acids in the aqueous phase of stool of humans consuming a daily dose of 3×15 g native uncooked high-amylose corn starch. In addition, an increase in primary bile acid excretion was observed in those volunteers consuming RS. Consequently, the cytotoxicity of the faecal aqueous phase decreased. Brown (1996) confirmed this. Adding 20 % crude potato starch to a hypercholesterolaemic rat diet also resulted in lower plasma cholesterol concentrations (–40 %) due to enhanced cholesterol and bile acid excretion (Levrat, 1996). Besides, Verbeek *et al*. (1995) suggested that the cholesterol-lowering effect of retrograded high-amylose corn starch in rats may be explained by an increased influx of neutral steroids and bile acids into the caecum, an increased faecal excretion of bile acids, and/or an altered bile acid profile.

The observed reduction of plasma cholesterol through consumption of RS shown in the above-mentioned studies was not confirmed in the present experiment, neither for normo- nor for hypercholesterolaemic rats. This discrepancy may be due to the fact that in the present study conventional instead of semi-synthetic diets were used. Adding RS to a conventional diet resulted in lower free, esterified and total plasma cholesterol concentrations when compared with results obtained in our previous study in which rats were fed on the same sources of RS types 2 and 3 added to a semi-synthetic diet (Vanhoof & De Schrijver, 1997). These semi-synthetic diets contained corn starch, beef tallow, casein, sucrose, and vitamins and minerals as the main ingredients (60, 14, 17·8, 3·8 and 4·5 % respectively). The RS types 2 and 3 sources replaced normal corn starch for 10·8 and 14·6 % in the test diets containing RS, whereas 1 % cholesterol and 0·1 % cholic acid replaced beef tallow in the hypercholesterolaemic diets.

Feeding semi-synthetic diets also resulted in liver total cholesterol concentrations approximately four times higher than the value in the present study for hypercholesterolaemic rats receiving the conventional diet. These observations can be caused by the lower digestibility of conventional diets compared with semi-synthetic diets. In addition, fermentation processes are less intensive when consuming conventional diets in comparison with semi-synthetic diets, since the effect of RS added to the conventional diet on caecal pH was less pronounced than for semi-synthetic diets. Also, adding RS to the conventional diet had no effect on caecal propionic and butyric acid concentrations whereas addition to a semi-synthetic diet significantly increased both values (results not published). Moreover, faecal cholesterol excretion was lower in rats receiving the normo- and hypercholesterolaemic semi-synthetic diets. Conversely, faecal excretion of coprostanol was higher under the same circumstances. Of the cholesterol excreted, 70 and 39 % was converted into coprostanol in rats fed on the semi-synthetic diets, whereas feeding conventional diets resulted only in 43 and 12 % conversion in normo- and hypercholesterolaemic rats respectively. Moreover, adding RS to hypercholesterolaemic semi-synthetic diets caused significantly higher coprostanol excretion and significantly lower total plasma cholesterol concentration. This significant effect was not confirmed in the present study. However, plasma cholesterol concentrations decreased by about 4 % and coprostanol excretion increased by 19 and 40 % when RS types 2 and 3 respectively were added to the hypercholesterolaemic conventional diet.

Higher daily excretion of bile acids was observed when conventional diets were fed rather than semi-synthetic diets. The effect on total 3α-hydroxy-bile acid excretion was more pronounced in normo- than in hypercholesterolaemic animals. The faecal primary:secondary bile acid ratio was lower in rats fed on the semi-synthetic diets, indicating that proportionally less primary bile acids were converted to secondary bile acids due to less intensive fermentation processes in the large bowel when conventional diets were fed.

The tendency to higher faecal bile acid excretion and the lower liver cholesterol concentration in rats receiving the conventional diets supplemented with RS had no influence on plasma cholesterol concentration. This has also been observed in studies with other fibre sources (Ghoos *et al*. 1988; Illman *et al*. 1991).

Care must be taken in interpreting these results as not only the type and dose of the RS source added to the diet but also the type of dietary fat influences the effect of RS on cholesterol metabolism (de Deckere *et al*. 1993, 1995). Moreover, it is well known that the level of food intake can affect plasma cholesterol concentrations. Consequently, in some studies it is not clear whether RS as such is responsible for the cholesterol-lowering effect or not (de Deckere *et al*. 1993). Addition of 1 % cholesterol and 0·1 % cholic acid to the rat diet might in itself cause liver pathology (Dabai *et al*. 1996) and, consequently, it may be useful to evaluate this rat model to assess the hypocholesterolaemic effects of foods.

This study clearly indicates that the consumption of a conventional diet supplemented with RS has a different influence on cholesterol metabolism compared with semi-synthetic diets. As a consequence, results obtained with semi-synthetic diets should be considered with caution as some metabolic effects might be affected by this particular dietary composition. Besides, the effect of RS added to the conventional diet might be masked due to the high levels of total dietary fibre in these diets (Table 1) which is much greater than the RS added. When considering the effect of RS consumption in humans, where dietary fibre intake is often very low, this overlapping of fibre and RS must be taken into account. Consequently, RS intake might influence cholesterol metabolism in humans taking a low-fibre diet.

Although the present data showed no significant effect of RS feeding on cholesterol metabolism in rats, these results may not reflect its effect in humans, particularly as rats have a different bile acid profile from humans (Heuman, 1989).

As a consequence, further research is necessary to find out how cholesterol metabolism might be influenced in different species, including humans.

References

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