Dietary rhubarb (*Rheum rhaponticum*) stalk fibre stimulates cholesterol 7α-hydroxylase gene expression and bile acid excretion in cholesterol-fed C57BL/6J mice

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Both experimental and clinical studies have indicated that a novel source of dietary fibre, produced from rhubarb (*Rheum rhaponticum*) stalks, is potentially hypolipidaemic. The present study, using C57BL/6J mice, was undertaken to examine if this fibre source affects cholesterol degradation. Mice were maintained on semi-purified diets containing 50 g rhubarb fibre or cellulose/kg with or without 5 g cholesterol/kg for 4 weeks. In cholesterol-supplemented mice, rhubarb fibre caused significant lowering of plasma cholesterol (−13 %) and the hepatic concentrations of total cholesterol (−34 %) and cholesteryl esters (−34 %). In parallel to the reduction of hepatic cholesteryl ester content, animals fed on rhubarb fibre had significantly lower activity of acyl CoA: cholesterol acyltransferase (*EC* 2.3.1.26) than the mice maintained on a diet containing cellulose and cholesterol. Rhubarb-fibre feeding accelerated the faecal bile-acid loss and diminished the gall-bladder bile-acid pool in both the normal and the cholesterol-fed mice. The increase in the bile-acid excretion was positively correlated with an increased activity as well as mRNA abundance of cholesterol 7α-hydroxylase (*EC* 1.14.13.17). The increased excretion of bile acids and induction of cholesterol 7α-hydroxylase activity may account for the hypocholesterolaemic effect of rhubarb fibre.

Rhubarb (*Rheum rhaponticum*): Bile acids: Cholesterol 7α-hydroxylase

Epidemiological studies have established a link between dietary fibre consumption and serum cholesterol concentrations. Fibres consisting of predominantly water-soluble components have proved to be more effective than insoluble types of fibre as hypocholesterolaemic agents (Anderson *et al.* 1990; Topping, 1991). Several mechanisms have been suggested for the cholesterol-lowering effects of these fibre sources, including disruption of micelle formation due to viscosity leading to lipid malabsorption (Gallaher *et al.* 1993), suppression of hepatic sterol synthesis by fermentation products of dietary fibre (Kishimoto *et al.* 1995), and binding of bile acids disrupting the enterohepatic recirculation of bile acids ultimately leading to an increase in faecal bile acid excretion (Fernandez, 1995; Matheson *et al.* 1995).

Rhubarb (*Rheum rhaponticum*) stalk powder, a predominantly insoluble fibre source, was recently found to depress the plasma cholesterol concentrations in experimental animals fed on high-cholesterol diets (Basu *et al.* 1993) and in hypercholesterolaemic subjects involved in a clinical trial (Goel *et al.* 1997). The mechanism by which this effect was achieved is unknown. Feeding rhubarb fibre to mice, however, has been shown to have no effect on the hepatic hydroxymethylglutaryl-CoA reductase (*EC* 1.1.1.34) activity (Basu *et al.* 1993) and hence it is unlikely that the hypocholesterolaemic action of the fibre source is mediated by inhibition of hepatic cholesterol synthesis.

Degradation of cholesterol to bile acids is the major pathway by which cholesterol is eliminated from the body and cholesterol 7α-hydroxylase (*EC* 1.14.13.17) is the rate-limiting enzyme in this process (Russel & Setchell, 1992). Although the mechanisms involved in the regulation of cholesterol 7α-hydroxylase are not fully understood, several studies have demonstrated that the cholesterol 7α-hydroxylase activity is induced by dietary cholesterol and the enzyme is subject to feedback inhibition by bile acids returning to the liver via the enterohepatic circulation (Russel & Setchell, 1992). Thus, decreased absorption of bile acids due to strong binding by cholestyramine increases cholesterol 7α-hydroxylase activity and mRNA 3–4-fold (Horton *et al.* 1994). The bile-acid-binding capacity of

Abbreviations: ACAT, acyl CoA: cholesterol acyltransferase.

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rhubarb stalk fibre has also been demonstrated in an *in vitro* study (Goel *et al.* 1998). It is, therefore, probable that the fibre exerts hypocholesterolaemic effects by interfering with the enterohepatic circulation of bile acids.

The present study was undertaken to determine the effects of rhubarb stalk fibre on faecal and biliary concentrations of bile acids in mice fed on diets with or without dietary cholesterol and to investigate if these changes correlated with the changes in the activity of cholesterol 7α-hydroxylase and its gene expression.

Materials and methods

Rhubarb fibre

Rhubarb stalks, both fresh and frozen, were obtained from small growers and food stores in Edmonton, Alberta. The stalks were cut into pieces of 30 mm length and steam-cooked for 10 min before squeezing them with a manual screw press to remove the juice. The pressed stalks were leached twice in about ten times their weight of water at 70° for 15 min and squeezed before being dried in a fluidized bed dryer at 85° for 1 h 40 min. Subsequently, the dry stalks were ground finely in a coffee grinder. The ground fibre was sifted through a 16 mesh sieve, analysed for chemical composition, packed in polyethylene bags and stored at room temperature. Table 1 gives the chemical composition of rhubarb stalk fibre.

Materials

[^{14}C]Palmitoyl CoA (2035 MBq/mmol) was obtained from American Radiolabeled Chemicals (St Louis, MO, USA) and [^{32}P]UTP was purchased from New England Nuclear (Boston, MA, USA). Cholesterol oxidase (*EC* 1.1.3.6) was obtained from Boehringer Mannheim Canada Ltd (Laval, Quebec, Canada; catalogue no. RC 393924).

Animals and diets

Male C57BL/6J mice (obtained from Jackson Laboratory, Basharbour, ME, USA), 8 weeks old, weighing 16–20 g were used (Paigen *et al.* 1987). Mice were housed (two per cage) in hanging stainless steel cages in a well-ventilated room maintained at 21 ± 2° with a 12 h light–dark cycle. All animals were fed on a pelleted diet (Purina Lab Rodent diet no. 5001; Purina, Richmond, IN, USA) for 1 week before being fed on an experimental semi-purified diet (Table 2). Animals were randomly divided into four groups of six animals each. The groups received diets containing either cellulose or rhubarb fibre (50 g/kg) with or without 5 g cholesterol/kg added at the expense of maize starch. The animal protocol of the study was approved by the University of Alberta Animal Welfare Committee.

Table 1. Chemical composition of rhubarb stalk fibre

(Values are means of two to four replicates)

Component	g/kg dry wt
Protein	56
Ash	56
Calcium	20
Oxalic acid	57
Malic Acid	32
Insoluble dietary fibre	659
Soluble dietary fibre	82
Total dietary fibre	741

Table 2. Composition (g/kg) of the semi-purified diets containing rhubarb stalk fibre (Rhub) or cellulose (Cellu) with and without cholesterol (C)

	Diets			
Ingredients*	Rhub	Cellu	Rhub + C	Cellu + C
Casein	200	200	200	200
Maize starch	595	595	590	590
Olive oil	100	100	100	100
Cholesterol	0	0	5	5
Vitamin mix†	20	20	20	20
Mineral mix‡	35	35	35	35
Cellulose powder	0	50	0	50
Rhubarb stalk powder§	50	0	50	0

* Ingredients were from ICN Biomedicals, Cleveland, OH, USA.

† AIN vitamin mix providing (mg/kg diet): retinyl acetate 19·8, ergocalciferol 1·38, DL-α-tocopheryl acetate 110, ascorbic acid 495, inositol 55, choline 2227, menadione 24·7, *p*-aminobenzoic acid 55, niacin 46·7, riboflavin 11, pyridoxine HCl 11, thiamin HCl 11, D-calcium pantothenate 33, biotin 0·2, pteroylmonoglutamic acid 0·99, cyanocobalamin 0·015.

‡ AIN mineral mix providing (g/kg diet): calcium phosphate dibasic 15, sodium chloride 2·2, potassium citrate monohydrate 6·6, potassium sulfate 1·56, magnesium oxide 0·7, manganous carbonate 0·105, ferric citrate 0·18, zinc carbonate 0·048, cupric carbonate 0·009, potassium iodate 0·0003, chromium potassium sulfate 0·0165.

§ Rhubarb stalk powder was prepared by blanching, drying and grinding the fresh rhubarb stalks. The powder contained 740 g total dietary fibre/kg with 660 g insoluble and 80 g soluble fibre/kg.

Sample collection

Body weight and daily food intake of all the animals were recorded once weekly. Two 24 h faecal samples were collected from each animal towards the end of the feeding period and were stored at −40° until analysis. After 4 weeks of dietary treatment the animals were fasted overnight and anaesthetized with halothane vapour for sample collection. Blood was drawn by cardiac puncture into plastic tubes containing anticoagulant (2·2 mmol EDTA dipotassium salt/l blood) and centrifuged at 1200 ***g*** for 20 min at 4° to obtain plasma. Livers were excised, blotted, weighed and quickly frozen in liquid N_2. Gall-bladder bile was obtained by aspiration using a syringe with 25 gauge needle. Separated plasma, gall-bladder bile and livers were stored at −80° until analysis.

Lipid analyses

Plasma total cholesterol was determined in duplicate by enzymic kit obtained from Sigma Biochemical (St Louis, MO, USA; catalogue no. 352-3). Free cholesterol levels in plasma were analysed by enzymic kit obtained from Boehringer Mannheim Canada Ltd (catalogue no. 139050) lacking esterase. Cholesterol esters were estimated as the difference between the total and the free cholesterol. The

total lipids from 50 mg liver samples were extracted using the chloroform–methanol (2:1, v/v) extraction procedure of Folch *et al.* (1957). The extract was evaporated to dryness under N_2 and then resuspended in 100 μl isopropanol. The concentrations of total and free cholesterol were determined using the enzymic kits as described previously. For the determination of faecal bile-acid levels, a 200 mg faecal sample from each animal was freeze-dried and ground, and steroids were extracted using ethanol and petroleum ether extraction (Malchow-Moller *et al.* 1982). The extract was dried under N_2 and resuspended in 1 ml methanol. Total bile acids were measured enzymically in a 20 μl extract by the 3α-steroid dehydrogenase method (Sigma Diagnostic Canada, Oakville, Ontario, Canada; catalogue no. 450-A). Gall-bladder bile (2 μl) was diluted to 100 μl with deionized double-distilled water, and bile acids, in 10 μl of the diluted bile, were quantified using the enzymic kit as stated earlier (Cheema *et al.* 1997).

Cholesterol 7α-hydroxylase activity and mRNA abundance

Samples of frozen livers (200–300 mg) were homogenized in ice-cold buffer containing 0·3 M-sucrose, 1 mM-EDTA, 50 mM-KCl, 0·1 M-K_2HPO_4 (pH 7·4) with five strokes of a Potter-Elvenhjem tissue homogenizer. Homogenates were centrifuged at 10 000 rev./min at 4° for 20 min to remove the cell debris. The supernatant fraction was recentrifuged for 70 min at 35 000 rev./min in a SW-60 rotor (Beckman Instruments, Palo Alto, CA, USA) at 4°. The microsomal pellet obtained from the second spin was resuspended in 500 μl buffer containing 0·1 M-K_2HPO_4 (pH 7·4), 1 mM-EDTA, 50 mM-KF, 5 mM-1,4-dithiothreitol and 50 mM-KCl. Portions of liver microsomes were then quickly frozen in liquid N_2 and stored at −80° until analysis. The cholesterol 7α-hydroxylase activity was measured by HPLC following a previously described method (Cheema *et al.* 1997).

Total RNA from mouse livers was purified according to standard procedures (Chomczynski & Sacchi, 1987). Cholesterol 7α-hydroxylase mRNA levels were determined by a ribonuclease protection assay. Total RNA (20 μg) was hybridized with ^{32}P-labelled antisense probes for mouse cholesterol 7α-hydroxylase (71 nt from intron 2 and 228 nt from exon 3 of the mouse cholesterol 7α-hydroxylase gene) (Tzung *et al.* 1994) and mouse glyceraldehyde-3-phosphate dehydrogenase (*EC* 1.2.1.12) synthesized from pTRI-glyceraldehyde-3-phosphate dehydrogenase (Ambion; Austin, TX, USA), at 55° overnight. Unhybridized probes were removed by treatment with RNase-1 (Promega Biotech; Madison, WI, USA) (4 U/μg RNA) for 1 h at 25°. The protected mRNA fragments were separated on 50 g/l polyacrylamide sequencing gels. The radioactivity in each band was quantitated by phosphorautoradiography using a Fuji-X BAS 1000 plate imager (Fuji, Richmond, Canada). The amount of cholesterol 7α-hydroxylase was normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA content.

Acyl CoA: cholesterol acyltransferase (ACAT) assay

ACAT was assayed by the method of Spector *et al.* (1980). Briefly, 200 μg microsomal protein was preincubated in 500 μl buffer containing 0·1 M-K_2HPO_4 (pH 7·2) and 1 mM-1,4-dithiothreitol for 5 min at 37°. The reaction was started by the addition of 10 nmol [1-^{14}C]palmitoyl CoA (1·85 kBq). Incubations were carried out for 5 min at 37° with shaking and the reaction was terminated with the addition of 2 ml chloroform–methanol (2:1, v/v). Lipids were extracted in the chloroform phase by the extraction procedure of Folch *et al.* (1957). Cholesteryl esters produced as a result of ACAT action were separated by TLC on silica gel-G plates, using a solvent system of light petroleum–diethyl ether–acetic acid (80:20:1, by vol.). The bands across the cholesteryl esters were scraped and radioactivity was counted in a liquid scintillation counter. From the known specific activity of [1-^{14}C]palmitoyl CoA substrate, ACAT activity was expressed as pmol cholesterol palmitate formed/min per mg microsomal protein.

Statistical analysis

The data were analysed using Statistical Analysis Systems version 11.0 (SAS Inc., Cary, NC, USA). A two-way ANOVA was employed with dietary cholesterol and dietary fibre as main effects. ANOVA was followed by Fisher's least significant difference test to compare treatment means. Differences were considered to be statistically significant if the associated *P* value was <0·05 (Steel & Torrie, 1980).

Results

Effect of rhubarb stalk fibre on plasma cholesterol

Table 3 shows that the mice fed on diets containing 50 g rhubarb fibre or cellulose/kg with or without 5 g

Table 3. Effect of feeding a diet containing either rhubarb stalk fibre (Rhub) or cellulose (Cellu) (50 g/kg) for 4 weeks with or without supplemental cholesterol (C) (5 g/kg) on food intake, weight gain and faecal weights of mice*

(Mean values with their standard errors for six mice)

	Food intake (g/d)		Body-weight gain (g)		Liver weight (g)		Faecal weight (g/24 h)	
Diets	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cellu	3·58	0·32	4·05	0·34	0·98	0·03	0·28	0·04
Rhub	3·95	0·20	3·91	0·38	1·11	0·04	0·28	0·02
Cellu + C	3·81	0·21	3·89	0·51	1·15	0·07	0·28	0·03
Rhub + C	3·78	0·29	4·38	0·54	1·14	0·06	0·28	0·04

* For details of diets and procedures, see Table 2 and pp. 66–68.

Table 4. Effects of dietary rhubarb stalk fibre (Rhub) and cellulose (Cellu) (50 g/kg) on the plasma cholesterol levels in mice fed on diets with or without supplemental cholesterol (C) (5 g/kg) for 4 weeks*

(Mean values with their standard errors for six mice)

	TC (mmol/l)		Free-C (mmol/l)		CE (mmol/l)	
Diets	Mean	SEM	Mean	SEM	Mean	SEM
Cellu	2·12[a]	0·02	0·41[a]	0·02	1·71[a]	0·01
Rhub	2·22[a]	0·05	0·44[a]	0·02	1·72[a]	0·01
Cellu + C	2·03[a]	0·08	0·38[ab]	0·02	1·65[a]	0·01
Rhub + C	1·77[b]	0·06	0·33[b]	0·01	1·44[b]	0·01

TC, total cholesterol; CE, cholesteryl esters.
[a,b] Mean values within a column not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 2 and pp. 66–67.

cholesterol/kg did not differ in terms of their food intakes, body-weight gains, liver weights or faecal weights.

Plasma cholesterol responses of the animals fed on diets containing different dietary fibre sources with or without cholesterol are given in Table 4. No differences were observed in plasma cholesterol concentrations of mice fed on cellulose or rhubarb-fibre diets without supplemental cholesterol. In animals fed on diets with added cholesterol, the plasma concentrations of total cholesterol remained unchanged. The group receiving rhubarb fibre (50 g/kg) with 5 g cholesterol/kg, however, had significantly ($P < 0·05$) lower concentrations of plasma total cholesterol and cholesteryl esters than the groups receiving the diet containing cellulose (50 g/kg) with or without 5 g cholesterol/kg.

Effect of rhubarb stalk fibre on liver lipids

In mice fed on diets without cholesterol, hepatic concentrations of total cholesterol, cholesteryl esters and activity of the enzyme ACAT were similar in groups fed on the cellulose and rhubarb fibre diets (Table 5). Unlike plasma cholesterol, hepatic concentrations were significantly elevated in the presence of a cholesterol supplement. The magnitude of reduction was much less in the presence of the rhubarb fibre than the cellulose. The difference was even more pronounced for the content of cholesteryl esters than for total cholesterol. In cholesterol-fed animals, an upregulation of ACAT activity was also observed. These results were in parallel with the increase in cholesteryl ester concentrations of the liver. The increase, however, was much less in mice fed on rhubarb fibre than in those fed on cellulose.

Effect of rhubarb stalk fibre on biliary and faecal bile acids

Since degradation of cholesterol to bile acids is the major pathway by which cholesterol is eliminated from the body, the effects of rhubarb stalk fibre on the faecal and biliary concentrations of bile acids were determined (Table 6). Mice fed on diets without added cholesterol showed a trend towards greater faecal bile-acid loss compared with cellulose controls. Cholesterol supplementation of the diets caused an approximately 2·5-fold increase in the faecal bile-acid levels. Mice fed on rhubarb fibre with added cholesterol had significantly greater ($P < 0·05$) faecal bile-acid loss in comparison with cellulose controls. Mice maintained on rhubarb fibre displayed lower concentrations of biliary bile acids than cellulose-fed animals. The concentration of total bile acids in the bile tended to be increased in mice fed on cholesterol-enriched diets, reaching significance only in the cellulose-fed animals.

Effect of rhubarb stalk fibre on cholesterol 7α-hydroxylase activity and mRNA

The accelerating effect of rhubarb stalk fibre on faecal bile-acid excretion, in combination with its depressing effect on biliary bile acids, led us to examine its effects on the activity of hepatic microsomal cholesterol 7α-hydroxylase and its mRNA abundance. In mice fed on diets without added cholesterol no significant differences were found in the activity of cholesterol 7α-hydroxylase or its mRNA abundance. When mice were fed on cholesterol-rich diets the activity of cholesterol 7α-hydroxylase increased significantly in both groups. Mice fed on rhubarb fibre and supplemental cholesterol expressed significantly greater ($P < 0·05$) activity than the mice fed on cellulose and dietary cholesterol (Fig. 1). The diet-induced changes in

Table 5. Effect of feeding a diet containing either rhubarb stalk fibre (Rhub) or cellulose (Cellu) (50 g/kg) for 4 weeks with or without supplemental cholesterol (C) (5 g/kg) on hepatic cholesterol levels and acyl CoA: cholesterol acyltransferase (ACAT) activity in mice*

(Mean values with their standard errors for six mice)

	TC (μmol/g)		Free-C (μmol/g)		CE (μmol/g)		ACAT†	
Diets	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Cellu	8·1[a]	0·53	2·8[a]	0·55	5·9[a]	0·47	25·7[a]	3·14
Rhub	7·3[a]	0·66	2·7[a]	0·24	4·6[a]	0·72	23·5[a]	2·34
Cellu + C	48·7[b]	4·43	3·1[ab]	0·09	41·1[b]	4·99	41·9[b]	1·91
Rhub + C	32·3[c]	3·36	3·5[b]	0·17	27·1[c]	5·44	32·3[c]	2·32

TC, total cholesterol; CE, cholesteryl esters.
[a,b] Mean values within a column not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 2 and pp. 66–67.
† Units are pmol cholesteryl esters formed/mg microsomal protein per min.

Table 6. Effect of feeding a diet containing either rhubarb stalk fibre (Rhub) or cellulose (Cellu) (50 g/kg) for 4 weeks with or without supplemental cholesterol (C) (5 g/kg) on faecal and biliary concentrations of total bile acids in mice*

(Means values with their standard errors for six mice)

	Faecal bile acids (μmol/d)		Biliary bile acids (mmol/l)	
Diets	Mean	SEM	Mean	SEM
Cellu	0·4[a]	0·08	68·5[a]	5·5
Rhub	0·6[a]	0·06	39·4[b]	3·9
Cellu + C	1·2[b]	0·08	93·9[c]	12·0
Rhub + C	1·6[c]	0·21	82·9[a]	7·3

[a,b,c] Mean values within a column not sharing a common superscript letter were significantly different, $P < 0·05$.
* For details of diets and procedures, see Table 2 and pp. 66–67.

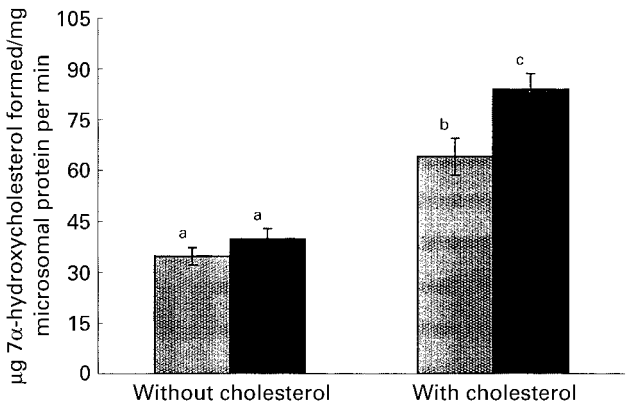

Fig. 1. Effects of dietary rhubarb stalk fibre (■) and cellulose (▨) (50 g/kg) on the activity of cholesterol 7α-hydroxylase in mice fed on diets with or without supplemental cholesterol (5 g/kg) for 4 weeks. Values are means for six animals, with their standard errors indicated by vertical bars. Bars not sharing a common superscript letter were significantly different, $P < 0·05$.

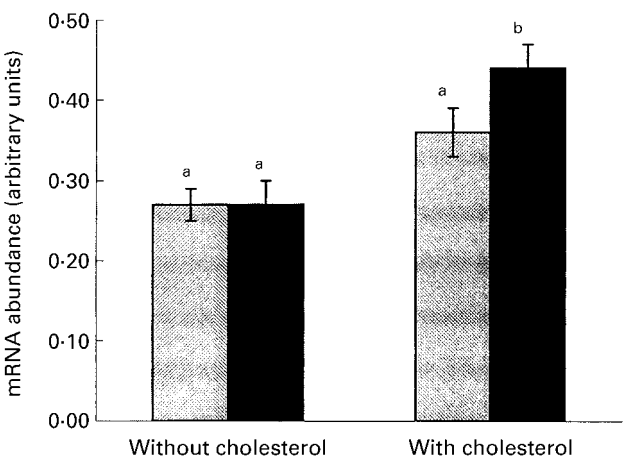

Fig. 2. Effects of dietary rhubarb stalk fibre (■) and cellulose (▨) (50 g/kg) on the mRNA abundance of cholesterol 7α-hydroxylase in mice fed on diets with or without supplemental cholesterol (5 g/kg) for 4 weeks. Values are means for six animals, with their standard errors indicated by vertical bars. Bars not sharing a common superscript letter were significantly different, $P < 0·05$.

cholesterol 7α-hydroxylase activity were accompanied by parallel changes in mRNA levels. The relative abundance of 7α-hydroxylase mRNA tended to increase when mice were fed on cholesterol-rich diets, but the effects were significant only in rhubarb-fed animals (Fig. 2).

Discussion

Both animal (Basu *et al.* 1993) and clinical (Goel *et al.* 1997) studies have previously suggested that rhubarb stalk fibre can potentially be a hypocholesterolaemic agent. In line with these observations the cholesterol-lowering effect of the fibre source was also evident in the present study. Significant reductions in hepatic total cholesterol and cholesteryl ester as well as plasma cholesterol concentrations were achieved in male C57BL/6J mice fed for 4 weeks on a diet containing 50 g rhubarb fibre/kg plus 5 g cholesterol/kg, compared with a diet where rhubarb fibre was replaced by cellulose. The C57BL/6J strain is generally considered to be hypersensitive to dietary cholesterol (Paigen *et al.* 1987), and yet feeding this strain of mice on a diet containing 5 g cholesterol/kg for 4 weeks failed to raise their plasma cholesterol levels. These somewhat unexpected results may be a reflection of the fact that the animals were sampled during overnight fasting, minimizing the levels of circulatory chylomicrons. It should also be pointed out that while the plasma levels of cholesterol remained unaffected, hepatic cholesterol concentrations were markedly increased following cholesterol supplementation, indicating hypercholesterolaemia. The cholesterol status of mice fed on a diet containing no added cholesterol remained unaffected by rhubarb stalk fibre, whereas mice fed on the cholesterol-enriched diet and rhubarb fibre showed marked reductions in both plasma and hepatic cholesterol levels. Why rhubarb stalk fibre did not affect cholesterol status in the absence of cholesterol supplementation cannot be explained at present. However, similar observations have been made by others who found that the cholesterol-lowering effect of dietary fibres such as psyllium (Horton *et al.* 1994) and non-starch polysaccharides (Abbey *et al.* 1993) were greater in the presence of dietary supplementation with cholesterol than without the supplementation. These fibres increased the LDL-receptor expression in guinea-pigs when fed together with high dietary cholesterol. Consequently, this effect may have resulted in a decreased hepatic synthesis of cholesterol and an increased uptake and catabolic rate of LDL, thus lowering plasma cholesterol concentrations.

The cholesterol-lowering effect of rhubarb stalk fibre when fed with dietary cholesterol was accompanied by an increased loss of bile acids and a decreased pool of biliary bile acids. Removal of excess cholesterol from the body is accomplished by secretion of cholesterol directly into the bile or after conversion to bile acids in the liver (Russel & Setchell, 1992). The biliary bile acids are released into the intestine from where they could be excreted into the faeces or reabsorbed, either passively or actively through ileal bile acid transporters, then recirculated to hepatocytes through the portal circulation. Anion exchange resins and bile-acid sequestrants such as cholestyramine and colestipol, and inhibitors of ileal bile-acid transporters such as

compound 2164U90, have been shown to increase faecal bile acid excretion and are associated with a concomitant reduction in the biliary bile-acid pool (Lewis *et al.* 1995). The ability of rhubarb stalk fibre to bind bile acids has been demonstrated in an *in vitro* study (Goel *et al.* 1998). This may result in an interruption of the enterohepatic bile acid circulation, and consequently this may lead to an increased diversion of cholesterol to bile acid synthesis in the liver, upregulation of lipoprotein receptors and depression of plasma cholestrol concentrations (Russel & Setchell, 1992). In contrast to rhubarb stalk fibre, dietary cellulose did not show any appreciable *in vitro* binding with bile acids such as taurocholate (Goel *et al.* 1998). In addition, feeding cellulose neither promoted faecal losses of bile acids nor led to a decrease in biliary bile acid concentration. Insoluble fibres are non-viscous and non-fermentable and, hence, they have a limited capacity to lower plasma cholesterol concentrations (Zacour *et al.* 1992). Although rhubarb extract is predominantly an insoluble fibre source (66 %), it contains pectin (8 %) which is a soluble fibre with a known cholesterol-lowering capacity (Fernandez *et al.* 1994; Fernandez, 1995). Furthermore, rhubarb extract contains malic acid and oxalic acid, which have not been studied in relation to their interaction with cholesterol absorption. These constituents may make rhubarb fibre a better cholesterol-lowering agent than cellulose.

In mice fed on diets containing rhubarb fibre and added cholesterol the increased faecal loss of bile acids and diminished gall-bladder bile-acid pool were accompanied by a small but significant increase in the activity of cholesterol 7α-hydroxylase, the regulatory enzyme in bile acid synthesis. Since the hepatic microsomal enzyme is regulated at pre-transcriptional level (Russel & Setchell, 1992), this may explain the positive correlation between the enzyme and its mRNA abundance found in the present study. The major form of regulation of cholesterol 7α-hydroxylase is the feedback inhibition of its gene transcription by hydrophobic bile acids in the enterohepatic circulation (Russel & Setchell, 1992). Thus, a reduction in the circulation of bile acids by administering bile acid sequestrants results in diminution of the biliary pool of bile acids and an increased expression of the cholesterol 7α-hydroxylase gene (Horton *et al.* 1994). The ability of rhubarb fibre to bind bile acids has been documented *in vitro* (Goel *et al.* 1998) and its feeding appears to decrease the biliary bile-acid pool. It is possible that the fibre source interferes with the absorption and enterohepatic cycling of bile acids *in vivo* and thereby the fibre counteracts the suppressive effect of bile acids on cholesterol 7α-hydroxylase expression, stimulating cholesterol degradation. Other fibres such as psyllium (Horton *et al.* 1994), pectin and guar gum (Fernandez, 1995) have also been shown to have stimulatory effects on cholesterol 7α-hydroxylase gene expression and its activity. As expected, cholesterol enrichment of the diets in the present study caused a significant increase in the activity of ACAT, the enzyme that catalyses the conversion of free cholesterol to cholesteryl esters in the tissues (Grogan *et al.* 1991). The increase, however, was much more pronounced in the cellulose group than in the rhubarb group, leading to a greater hepatic concentrations of cholesteryl ester in mice fed on cellulose and cholesterol. The increased bile-acid excretion due to rhubarb fibre feeding might have increased the conversion of cholesterol to bile acids, resulting in a reduction of the hepatic storage of cholesterol.

In conclusion, the results of the present study suggest that rhubarb fibre reduces plasma and liver cholesterol concentrations in cholesterol-fed mice. The effect is probably due to its ability to bind bile acids (Goel *et al.* 1998), consequently increasing their faecal excretion and diminishing the biliary bile-acid pool. These effects may have led ultimately to an induction of the cholesterol 7α-hydroxylase gene and an increase in cholesterol degradation. Thus, rhubarb stalk fibre appears to have the potential to be an effective cholesterol-lowering fibre source.

References

Abbey M, Triantafilidis C & Topping DL (1993) Dietary non-starch polysaccharides interact with cholesterol and fish oil in their effects on plasma lipids and hepatic lipoprotein receptor activity in rats. *Journal of Nutrition* **123**, 900–908.

Anderson JW, Deakins DA, Floore TL, Smith BM & Whitis SE (1990) Dietary fiber and coronary heart disease. *Critical Reviews in Food Science and Nutrition* **29**, 95–136.

Basu TK, Ooraikul B & Garg M (1993) The lipid lowering effects of rhubarb stalk fiber: a new source of dietary fiber. *Nutrition Research* **13**, 1017–1024.

Cheema SK, Cikaluk D & Agellon LB (1997) Dietary fats modulate the regulatory potential of dietary cholesterol on cholesterol 7α-hydroxylase gene expression. *Journal of Lipid Research* **38**, 315–323.

Chomczynski P & Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162**, 156–158.

Fernandez ML (1995) Distinct mechanisms of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum and psyllium. *Journal of Lipid Research* **36**, 2394–2404.

Fernandez ML, Sun DM, Tosca MA & McNamara DJ (1994) Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipid metabolism: a dose response study in guinea pigs. *American Journal of Clinical Nutrition* **59**, 869–878.

Folch J, Lees M & Sloane-Stanley S (1957) A simple method for the isolation and purification of total lipides from animal sources. *Journal of Biological Chemistry* **226**, 497–509.

Gallaher DD, Hassel CA, Lee K & Gallaher C (1993) Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effect of hamsters. *Journal of Nutrition* **123**, 244–252.

Goel V, Cheema SK, Agellon LB, Ooraikul B, McBurney MI & Basu TK (1998) In vitro binding of bile salt to rhubarb stalk powder. *Nutrition Research* **18**, 893–903.

Goel V, Ooraikul B & Basu TK (1997) Cholesterol lowering effects of rhubarb fiber in hypercholesterolemic men. *Journal of the American College of Nutrition* **16**, 600–604.

Grogan WM, Bailey ML, Hueman DM & Vhahcevic ZR (1991) Effects of perturbations in hepatic free and esterified cholesterol pools on bile acid synthesis, cholesterol 7α-hydroxylase, HMG CoA-reductase, acyl CoA: cholesterol acyltransferase and cytosolic ester hydrolase. *Lipids* **26**, 907–914.

Horton JD, Cuthbert JA & Spady DK (1994) Regulation of hepatic 7α-hydroxylase expression by dietary psyllium in the hamster. *Journal of Clinical Investigation* **93**, 2084–2092.

Kishimoto Y, Wakabayashi S & Takida H (1995) Hypocholesterolemic effect of dietary fiber: relation to intestinal fermentation

and bile acid excretion. *Journal of Nutritional Science and Vitaminology* **41**, 151–161.
Lewis MC, Briecady LE & Root C (1995) Effect of 2164U90 on ileal bile acid absorption and serum cholesterol in rats and mice. *Journal of Lipid Research* **36**, 1098–1105.
Malchow-Moller A, Arffmann S, Larusso NF & Krag E (1982) Enzymatic determination of total 3α-hydroxy bile acids in faeces. *Scandinavian Journal of Gastroenterology* **17**, 331–333.
Matheson HB, Colon IS & Storey JA (1995) Cholesterol 7α-hydroxylase activity is increased by dietary modification with psyllium hydrocolloid pectin, cholesterol and cholestyramine in rats. *Journal of Nutrition* **125**, 454–458.
Paigen B, Holmes PA, Mitchell D & Albee D (1987) Comparison of atherosclerotic lesions and HDL lipid levels in male, female and testosterone treated female mice from strains C57BL/6, BALB/C and C3H. *Atherosclerosis* **64**, 215–221.
Russel DW & Setchell KDR (1992) Bile acid biosynthesis. *Biochemistry* **31**, 4737–4748.
Spector AA, Kaduce TL & Dane RW (1980) Effect of dietary fat saturation on acyl coenzyme: cholesterol acyltransferase activity of rat liver microsomes. *Journal of Lipid Research* **21**, 169–179.
Steel RGD & Torrie JH (1980) *Principles and Procedures of Statistics: A Biometrical Approach*, pp. 67–119. New York, NY: McGraw-Hill.
Topping DL (1991) Soluble fiber polysaccharides: effect on plasma cholesterol and colonic fermentation. *Nutrition Reviews* **49**, 195–203.
Tzung KW, Ishimura-Oka K, Kihara S, Oka K & Chan L (1994) Structure of the mouse cholesterol 7α-hydroxylase gene. *Genomics* **21**, 244–247.
Zacour AC, Silva ME, Cecon PR, Bambirra EA & Vieira EC (1992) Effect of dietary chitin on cholesterol absorption and metabolism in rats. *Journal of Nutritional Science and Vitaminology* **38**, 609–613.