Psyllium and fat in diets differentially affect the activities and expressions of colonic sphingomyelinases and caspase in mice

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Dietary fibre and fat affect colonic tumourigenesis and inflammation. Sphingomyelin metabolism may have implications for the pathogenesis of colonic tumours and ulcerative colitis. The present study examined the effects of psyllium and fat on the enzymes responsible for sphingomyelin metabolism and apoptosis in the colon. Mice were fed control, psyllium-containing (100 g/kg), high-fat (313 g/kg, 53 % energy as fat) or high-fat plus psyllium diets for 4 weeks. The activities of acid, neutral and alkaline sphingomyelinase (SMase), neutral ceramidase, and caspase 3, 8 and 9 in colonic mucosa were determined. The expressions of alkaline SMase and caspase 3 were examined. The psyllium-containing diet was found to increase significantly the activities of alkaline SMase and caspase 3 and decreased those of acid SMase and neutral ceramidase. The high-fat diet had opposite effects on these enzymes and attenuated the effects of psyllium. Western blotting showed that psyllium increased and high-fat decreased the levels of alkaline SMase and caspase 3 in colonic mucosa. The change in caspase 3 activity was positively correlated with that of alkaline SMase and negatively with acid SMase. No similar changes of acid and alkaline phosphatase activities in the colon or acid and neutral SMase activity in the liver were identified. In conclusion, colonic sphingomyelin metabolism and apoptosis were affected by psyllium and fat in an opposite manner. The results may have implications for colorectal tumourigenesis and inflammation.

Psyllium: Fat: Sphingomyelinase: Colon

Sphingomyelin (SM) is a type of sphingolipid. It is present in all eukaryotic cells and in dietary products such as milk, egg and meat (Zeisel *et al.* 1986; Blank *et al.* 1992). SM is hydrolysed sequentially by sphingomyelinase (SMase) and ceramidase to ceramide and sphingosine, which are considered to be major anti-proliferative molecules affecting tumourigenesis (Hannun & Bell, 1989; Kolesnick, 1991; Merrill *et al.* 1997). In the intestinal tract there are three types of SMase: acid, neutral and alkaline. Our previous studies have shown that alkaline SMase is specifically expressed in the intestinal mucosa and located on the surface of the brush border (Nilsson, 1969; Duan *et al.* 1995, 2003; Cheng *et al.* 2002). We also identified a neutral ceramidase in the gut that is distributed in parallel with alkaline SMase (Nilsson, 1969; Lundgren *et al.* 2001). The alkaline SMase and neutral ceramidase are the major enzymes that are responsible for digestion of sphingolipids in the gut (Nyberg *et al.* 1997; Duan *et al.* 2001).

Digestion of dietary SM may have clinical implications. Supplementary SM in the diet has been shown to inhibit colonic tumourigenesis in animals treated with 1,2-dimethylhydrazine (Dillehay *et al.* 1994). In the tissues of human adenomas, carcinomas and longstanding ulcerative colitis, the activities of alkaline SMase were decreased (Hertervig *et al.* 1996, 1999; Sjöqvist *et al.* 2002; Duan *et al.* 2003). The reduced alkaline SMase activity may affect SM digestion, decrease the level of ceramide and increase the susceptibility of colonic mucosa to carcinogenic factors.

It is well known that the development of colon cancer is determined by the interplay of genetic and environmental factors. The influence of dietary components, particularly fat and fibre, has gained great attention. Based on most (but not all) ecological and epidemiological studies, case–control and cohort studies, and studies of migrants and laboratory animals, it is generally suggested that a high-fat diet increases, and a high-fibre diet decreases, the risk of colon cancer. The effect of fat varies with the type of the fat and the composition of fatty acids: saturated fat is most noxious. The effects of fibre are also dependent on the type and the metabolism of the fibre in the gut. The detailed background of the effects of dietary fat and fibre on pathogenesis of colorectal cancer has been summarised in several excellent reviews (Lipkin *et al.* 1999; Kim, 2000; Reddy *et al.* 2003). In addition to effects on tumourigenesis, dietary factors may also affect the process of colonic inflammation. A high-fat diet has been reported to increase the risk of ulcerative colitis (Reif *et al.* 1997; Geerling *et al.* 2000) and dietary fibre may prolong the remission of the inflammation (Fernandez-Banares *et al.*

1999; Kanauchi *et al.* 2002). The mechanism underlying the effects of the dietary factors on colon cancer and intestinal bowel disease has not been well established, although the alterations of the colonic concentrations of bile salts, Ca and carcinogens may be involved, and the changed expression of Cox2 and *ras* have been suggested (Reddy *et al.* 2003). We have previously shown that the insoluble fibre cellulose in a high-fat diet influenced the activities of acid and neutral, but not alkaline, SMase (Yang *et al.* 2002). However, whether high dietary fat *per se* and whether other types of fibre affect SMase (particularly alkaline SMase) activity remains elusive.

Psyllium is a type of water-soluble fibre derived from *Plantago ovata*. Psyllium husks are pure dietary fibres, composed mostly of hemicellulose. They are not digested in the small intestine and are partially broken down in the colon, where they act as a food source for normal bacterial flora and increase the bulk of the stool. Psyllium has been used for intestinal health in Europe since the 16th century with few harmful side effects reported. Recently psyllium has been found to have anti-carcinogenic and anti-inflammatory effects in the colon (Robert-Andersen *et al.* 1987; Alabaster *et al.* 1993; Cohen *et al.* 1996; Fernandez-Banares *et al.* 1999). As SM metabolism has been found to be altered in both colorectal cancer and colitis, the present study was designed to study the effects of psyllium and fat on the activities and expression of the enzymes that are responsible for SM digestion and apoptosis in the colon.

Materials and methods

Materials

The experiment was approved by the Animal Ethics Committee of the Medical Faculty, Lund University. Mice (Balb/c) at the age of 4 weeks were obtained from M&B (Ry, Denmark). Purified psyllium was provided by Dr Falk Pharma GmbH (Freiburg, Germany). [14C]choline-labelled SM (2·1 MBq/mg) was provided by Dr Lena Nyberg (Skåne Dietary Association, Malmö, Sweden). Anti-human alkaline SMase antibody was developed in the laboratory as described by Duan *et al.* (2003) and anti-caspase 3 antibody was purchased

from Calbiochem (San Diego, CA, USA). The substrates of caspase 3 (aspartate-glutamate-valine-aspartate-pentose nucleic acid), caspase 8 (acetyl-isoleucine-glutamate-threonine-aspartate-*p*-nitroaniline) and caspase 9 (acetyl-leucine-glutamate-histidine-aspartate-pentose nucleic acid) were purchased from Upstate Biotech (Lake Placid, NY, USA). [14C]octanoic acid was purchased from American Radiolabeled Chemicals Inc (St Louis, MO, USA). The materials for preparation of experimental diets were obtained from ICN (Stockholm, Sweden), except butter, sunflower seed oil and rapeseed oil, which were purchased from the local supermarket.

Experimental diets and animals

Four types of semisynthetic diets were prepared. The control and high-psyllium (100 g/kg) diets were modified AIN-93G diets (Reeves *et al.* 1993) and were similar with regard to protein, fat and carbohydrate content. The high-fat diet was prepared by enriching the diet with butter and rapeseed oil, but still providing 3 % energy from sunflower seed oil to prevent symptoms of essential fatty acid deficiency (Pajari & Mutanen, 1999). The high-fat plus psyllium diet was prepared by addition of psyllium (100 g/kg) to the high-fat diet. The composition and the % macronutrients as energy of the experimental diets are shown in Tables 1 and 2. All the diets were designed to be comparable in terms of the amounts of protein and fat, both in relation to weight and energy content. The prepared diets were stored at −20°C and given daily in excess to the mice.

Forty-eight mice were divided into four groups and each group was given one type of diet for 4 weeks. The body weight of the animal was determined weekly. The mice were killed by cervical dislocation. The colon was removed and the colonic mucosa was scraped and homogenised as described by Duan *et al.* (1995). The homogenate was further sonicated for 10 s followed by centrifugation at 15 000 relative centrifugal force for 15 min. The supernatant fraction was saved for analysis of SMase and caspase. Four animals in each group were randomly selected for measurement of expression of the enzymes by Western

Table 1. Dietary composition (g/kg)

Components	Control	Psyllium	High-fat	High-fat + psyllium
Casein	217	196	278	250
Dextrose	360	324	209	188
Starch	294	265	139	125
Total carbohydrate	654	589	348	313
Butter	0	0	208	188
Sunflower seed oil	42	38	18	16
Rapeseed oil	33	30	86	78
Total fat	75	68	313	282
Psyllium	0	100	0	100
Mineral mix	368	33·2	41·6	37·4
Vitamin mix	10·5	9·5	11·8	10·6
L-Cystine	3·2	2·8	3·6	3·2
Choline chloride	3·2	2·8	3·6	3·2
BHT	0·14	0·14	0·14	0·14

BHT, butylated hydroxytoluene.

Table 2. Dietary composition (% energy)

Diet	Protein	Carbohydrate	Fat
Control	20·89	62·88	16·23
Psyllium	20·90	62·88	16·22
High-fat	20·90	26·16	52·93
High-fat + psyllium	20·90	26·16	52·94

blot before feeding. The livers in all animals were also removed and homogenised similarly.

Sphingomyelinase assay

The alkaline SMase activity was determined as described by Duan & Nilsson (1999). In brief, a 5 μl sample was added to 95 μl 50 mM-Tris-buffer containing 0·15 M-NaCl, 2 mM-EDTA, 6 mM-sodium taurocholate and 80 pmol [^{14}C]SM (133 Bq), pH 9·0. The reaction was stopped by the addition of 0·4 ml chloroform–methanol (2:1, v/v) followed by centrifugation at 10 000 relative centrifugal force for 10 s. A portion of the upper phase was taken and the production of [^{14}C]phosphocholine was determined by liquid scintillation. The activities of acid and neutral SMase were determined in a similar way in modified buffers. The acid SMase was assayed in 50 mM-maleate buffer containing 0·15 M-NaCl, Triton X100 (1·2 g/l), pH 5·0; neutral SMase was assayed in 50 mM-HCl buffer containing 4 mM-Mg^{2+} and Triton X100 (1·2 g/l), pH 7·5.

Synthesis of D-erythro-[^{14}C]octanoyl ceramide and ceramidase assay

1-[^{14}C]octanoyl ceramide was prepared according to Bielawska & Hannun (2000) with modification. [^{14}C]octanoic acid (3·8 μmol) was dried and reacted with 38 μmol octanoyl chloride in 0·5 ml benzene for 2 h. The solution was dried under N$_2$ and reacted with 25 mg D-erythrosphingosine in 2·5 ml tetrahydrofuran and 1·25 ml sodium acetate (500 g/l) for 4 h with stirring. The reaction was terminated by adding 6 ml chloroform, 3 ml methanol and 2·25 ml water. The labelled 1-[^{14}C]octanoyl ceramide present in the lower phase was purified by TLC and the plate (Whatman®, Maidstone, Kent, UK; 150 Å, 0·5 mm) was developed by chloroform–methanol–2 M-ammonium hydroxide (4·0:1·0:0·1, by vol.). The specific activity of the labelled ceramide was about 183 Bq/nmol.

For ceramidase assay, 1-[^{14}C]octanoyl ceramide was dried under N$_2$, suspended in 50 mM-Tris-maleate buffer, pH 7·0, containing 10 mM-sodium taurocholate (assay buffer), and sonicated for 2 min on iced water. A 10 μl sample was mixed with 90 μl assay buffer containing 2 nmol 1-[^{14}C]octanoyl ceramide, followed by incubation at 37°C for 1 h. The reaction was interrupted by addition of 0·6 ml methanol–chloroform–heptane (28:25:20, by vol.) and 0·2 ml 0·05 M-K$_2$CO$_3$/K$_2$B$_2$O$_2$, pH 10. After centrifugation at 10 000 relative centrifugal force for 10 s, 200 μl upper phase were taken for liquid scintillation counting. In the upper phase about 90 % of fatty acids was recovered under these conditions. This incomplete recovery was adjusted for in subsequent calculations (Lundgren et al. 2001).

Caspase assay

The activities of caspase 3, 8 and 9 were determined as described previously (Liu et al. 2002) in a buffer containing 50 mM-HEPES, 100 mM-NaCl, 3-((3-cholamidopropyl) dimethylammonio)-1-propane-sulfonate (1 g/l), 10 mM-dithiothrietol, 0·1 mM-EDTA, glycerol (100 g/l) and 2 mM specific substrates, pH 7·4. The cleavage of the substrates was determined by the absorbance at 405 nm on a microplate reader (Bio-Rad, Stockholm, Sweden) every 30 min up to 2 h, using p-nitroaniline as a standard. The activities were expressed as the production of p-nitroaniline/min per mg sample protein.

Western blotting

Western blotting of alkaline SMase was performed as described by Duan et al. (2003). Protein (50 μg) in each sample was resolved by SDS (100 g/l)-PAGE and transferred to nitrocellulose membrane electrophoretically. The membrane was probed with polyclonal rabbit anti-human alkaline SMase antibody (1:500 dilution) and then with goat anti-rabbit antibody conjugated with alkaline phosphatase. For Western blotting of caspase 3, 50 μg protein in each sample was subjected to SDS (150 g/l)-PAGE and then transferred to nitrocellulose membrane as described earlier. The membrane was then incubated with mouse anti-caspase 3 antibody (1:2000) overnight and then with goat anti-mouse antibody conjugated with alkaline phosphatase for 2 h. The bands were visualised by a kit obtained from Bio-Rad. The densities of the bands were quantified by Scion Image Software after transforming the files to TIF format. The program was obtained from Scion Co. (www.scioncorp.com).

Table 3. The body weight (g) of mice fed different types of diets for 4 weeks*†
(Mean values and standard deviations for twelve mice per group)

Diet	Week 0		Week 1		Week 2		Week 3		Week 4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	16·59	1·70	17·43	1·83	18·37	1·63	19·63	1·73	20·15	1·80
Psyllium	17·16	1·56	17·68	1·63	17·92	1·42	18·50	1·45	19·31	1·56
High-fat	16·68	1·45	17·44	1·56	18·62	1·73	19·50	1·80	20·08	1·73
High-fat + psyllium	16·83	0·87	17·82	0·90	18·82	0·69	19·38	0·87	20·22	0·73

* For details of diets and procedures, see Tables 1 and 2 and p. 716.
† The body weights were determined weekly.

Other biochemical analyses

The activities of acid and alkaline phosphatases were determined as described previously using *p*-nitrophenyl phosphate as a substrate (Cheng *et al.* 1999). The proteins in the homogenate were analysed using DC protein assay kit obtained from Bio-Rad.

Statistical analyses

Results are presented as mean values and standard deviations. The results from the psyllium and high-fat groups were compared with those from the control group. The results from the high-fat group were also compared with those from the high-fat plus psyllium group. The statistical significance ($P < 0.05$) of the difference for alkaline SMase and caspase activities between each comparison was determined by unpaired Student's *t* test. The results of Western blotting were non-parametric data and the significance of differences of the band density was determined by Wilcoxon–Mann–Whitney test. $P < 0.05$ was considered to be statistically significant.

Results

Changes in body weight

The changes in body weights of mice in each dietary group are shown in Table 3. There was no statistical significance in the animals' body weight in different groups during the experiment, although the gain in body weight of the mice in the psyllium group was less than those in other groups.

Changes of sphingomyelinase activities

The changes in alkaline, acid and neutral SMase activities induced by the diets are shown in Fig. 1. Compared with the control group, the psyllium-containing diet significantly increased the activity of alkaline SMase by 57 % ($P < 0.05$) and reduced that of acid SMase by 32 % ($P < 0.001$). Conversely, the high-fat diet sharply reduced the activity of alkaline SMase by 64 % ($P < 0.01$), but increased that of acid SMase by 13 % ($P < 0.05$). The effect of fat on alkaline SMase was partially reversed (and that on acid SMase was totally reversed) by addition of psyllium in the diet. Both high-fat and psyllium-containing diets did not affect neutral SMase activity.

Changes of caspase activities

The activities of caspase after feeding the diets are shown in Fig. 2. Compared with the control diet, the addition of 100 g psyllium/kg significantly increased the activity of caspase 3 by about 100 % ($P < 0.05$) and increased that of caspase 8 slightly (NS). The high-fat diet significantly reduced the activities of caspase 3, 8 and 9 by 73 ($P < 0.01$), 52 ($P < 0.05$) and 47 % ($P < 0.01$) respectively. The reductions caused by fat were partly reversed by psyllium in the high-fat plus psyllium diet.
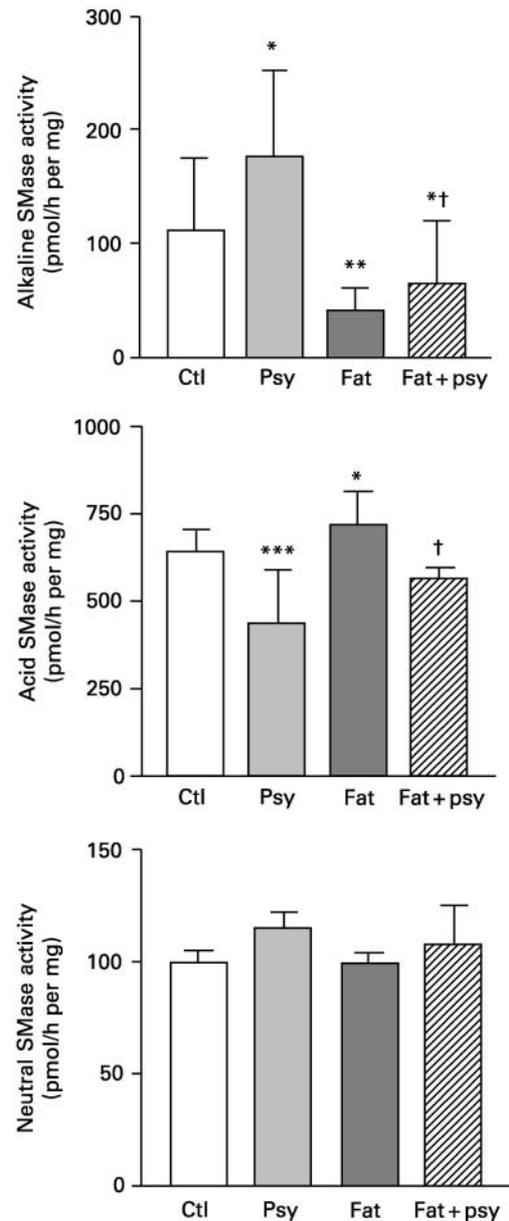

Fig. 1. Activities of sphingomyelinase (SMase) in colonic mucosa of mice (twelve per group) fed control (Ctl), psyllium-containing (Psy), high-fat (Fat) or high-fat plus psyllium (Fat + psy) diets for 4 weeks. The colonic mucosa was scraped and homogenised. The activities of acid, neutral and alkaline SMase were determined using [14C]choline-labelled sphingomyelin as substrate. For details of diets and procedures, see Tables 1 and 2 and pp. 716–717. Values are means with standard deviations shown by vertical bars. Mean values were significantly different from those of the control group: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Mean values were significantly different from those of the high-fat group:†$P < 0.05$.

Expressions of alkaline sphingomyelinase and caspase 3 by high-fat and psyllium-containing diets

To assess whether the changed activities of alkaline SMase and caspase 3 were associated with decreased expressions of the enzymes, Western blotting was performed in four randomly selected samples in each group. Fig. 3(a) shows representative results of Western blots and Fig. 3(b) shows the densities of alkaline SMase and caspase 3
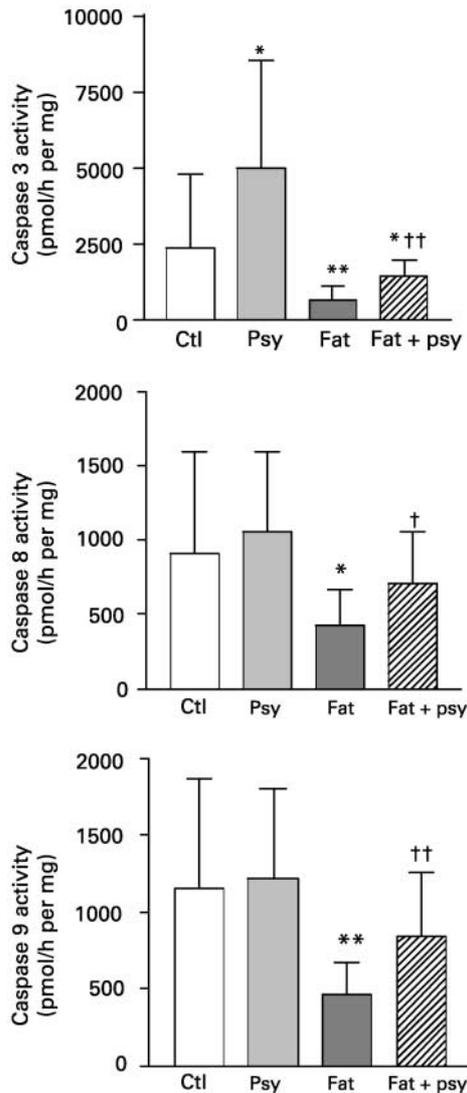
Fig. 2. Activities of caspases in colonic mucosa of mice (twelve per group) fed control (Ctl), psyllium-containing (Psy), high-fat (Fat) or high-fat plus psyllium (Fat + psy) diets for 4 weeks. The colonic mucosa was scraped and homogenised. The activities of caspase 3, 8 and 9 were determined using specific substrates. For details of diets and procedures, see Tables 1 and 2 and pp. 716–717. Values are means with standard deviations shown by vertical bars. Mean values were significantly different from those of the control group: $*P < 0.05$, $**P < 0.01$. Mean values were significantly different from those of the high-fat group: $†P < 0.05$, $††P < 0.01$.

Fig. 3. Expression of alkaline (alk) sphingomyelinase (SMase) and caspase 3 in mice (twelve per group) fed control (Ctl), psyllium-containing (Psy), high-fat (Fat) or high-fat plus psyllium (Fat + psy) diets for 4 weeks. The colonic mucosa was scraped and homogenised. Four animals in each group were randomly selected for Western blotting analysis. In each lane, $50\,\mu g$ sample proteins were loaded. For details of diets and procedures, see Tables 1 and 2 and pp. 716–717. (a), A representative result of Western blotting. Lane A, Ctl; lane B, Psy; lane C, Fat; lane D, Fat + psy. (b), Quantification of the band densities by Scion Image software (Scion Co.; www.scioncorp.com). Values are means with standard deviations shown by vertical bars. Mean values were significantly different from those of the control group: $*P < 0.05$.

bands in these samples. The levels of alkaline SMase were significantly increased by psyllium ($P < 0.05$) and decreased by the high-fat diet ($P < 0.05$). The caspase 3 levels were also significantly increased ($P < 0.05$) by the psyllium-containing diet. The levels of caspase 3 appeared to be reduced by the high-fat diet, although the changes were not statistically significant ($P = 0.057$, Wilcoxon–Mann–Whitney test). In Western blot for caspase 3 we detected the presence of pro-caspase 3 (Fig. 3 (a)), but not the cleaved active form.

Changes in neutral ceramidase activity

The effects of the diets on neutral ceramidase activity are shown in Fig. 4. In comparison with the control diet, the
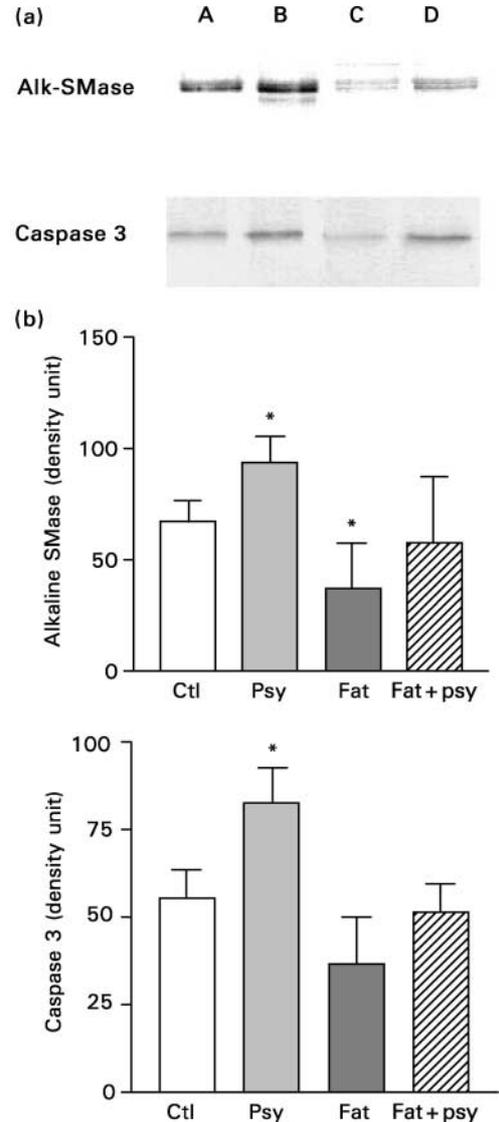
high-psyllium diet significantly reduced ceramidase activity by 50 % ($P < 0.001$) and the high-fat diet reduced ceramidase activity by about 30 % (NS). Combination of high-fat and psyllium gave a 62 % reduction in ceramidase activity ($P < 0.001$).

Correlation of caspase 3 activity with sphingomyelinase and ceramidase

To investigate whether there was a link between caspase 3 with any of the SMase and ceramidase, the correlation of
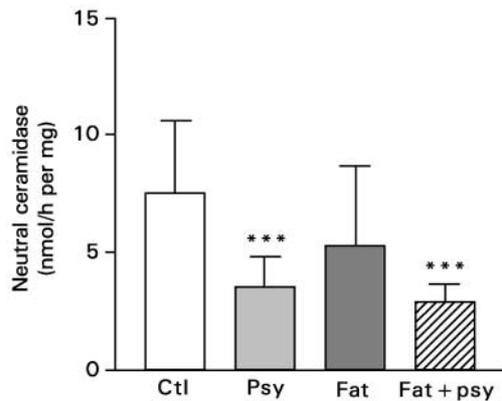
Fig. 4. Activities of neutral ceramidase in mice (twelve per group) fed control (Ctl), psyllium-containing (Psy), high-fat (Fat) or high-fat + psyllium (Fat + psy) diets for 4 weeks. The colonic mucosa was homogenised and the activity of neutral ceramidase was determined. For details of diets and procedures, see Tables 1 and 2 and pp. 716–717. Values are means with standard deviations shown by vertical bars. Mean values were significantly different from that of the control group: ***$P < 0.001$.

the activities of SMase and caspase 3 in all the animals was analysed and the results are shown in Fig. 5. The activity of caspase 3 was found to correlate positively with alkaline SMase ($P < 0.0001$) and negatively with acid SMase ($P < 0.0001$). Similar correlation of caspase 8 or 9 with alkaline and acid SMase was also identified (results not shown). However, there was no correlation between the activity of neutral SMase and any caspase. In addition, there was no correlation between ceramidase activity and caspase activities (results not shown).

Activities of acid and alkaline phosphatase in the gut and acid and neutral sphingomyelinase in the liver

To assess the specificity of the changes of SMase induced by the diets in the colon, the activities of acid and alkaline phosphatase in the colonic mucosa and those of acid and neutral SMase in the liver after feeding these diets were determined. As shown in Table 4, the activities of both acid and alkaline phosphatase were decreased by the high-fat diet ($P < 0.0001$ and $P < 0.005$ respectively). The decreases were not restored by addition of psyllium to the high-fat diet. Table 5 shows that these diets had no effect on either acid or neutral SMase activities in the liver.

Discussion

In the present work we demonstrated that a psyllium-containing and a high-fat diet affected the activities of the enzymes that are responsible for SM metabolism and apoptosis in the colon. The psyllium-containing diet increased the activities of alkaline SMase and caspase, and decreased those of acid SMase and neutral ceramidase. The high-fat diet gave, in general, effects opposite to those of psyllium, decreasing the activities of alkaline SMase and caspase and increasing that of acid SMase. The change of alkaline SMase correlated positively and that of acid SMase negatively with the activity of caspase 3, the key enzyme in execution phase of apoptosis.
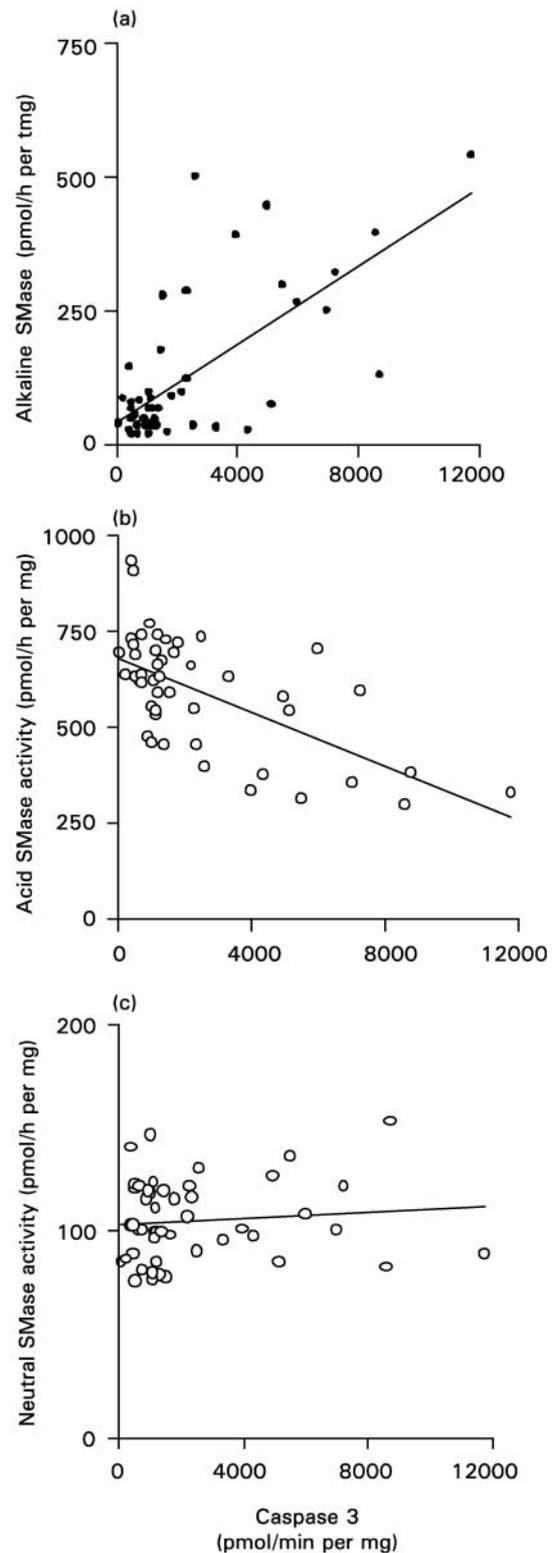

Fig. 5. Correlation of caspase 3 with acid, neutral and alkaline sphingomyelinase (SMase) in mice (twelve per group) fed control (Ctl), psyllium-containing (Psy), high-fat (Fat) or high-fat + psyllium (Fat + psy) diets for 4 weeks. The activities of acid, neutral and alkaline SMase and caspase 3 in the intestinal mucosa were determined. For details of diets and procedures, see Tables 1 and 2 and pp. 716–717. The correlations of caspase 3 activity with SMase were: (a), r^2 0.4735, $P < 0.0001$; (b), r^2 0.3914, $P < 0.0001$; (c), r^2 0.01072, $P = 0.4838$.

Table 4. The activities (unit/mg per h) of acid and alkaline phosphatases in colonic mucosa of mice fed various diets for 4 weeks†

(Mean values and standard deviations for twelve mice per group)

Diet	Acid phosphatase		Alkaline phosphatase	
	Mean	SD	Mean	SD
Control	1·321	0·232	0·816	0·678
Psyllium	1·247	0·215	0·353	0·498
High-fat	0·865**	0·090	0·102*	0·163
High-fat + psyllium	0·679**	0·080	0·022*	0·007

Mean values were significantly different from those of the control group: *P<0·005, **P<0·0001.

† The colonic mucosa was homogenised and the activities of acid and alkaline phosphatase were determined. For details of diets and procedures, see Tables 1 and 2 and p. 716.

Table 5. Acid and neutral sphingomyelinase activities (pmol/h per mg) in the liver in mice fed various diets for 4 weeks*

(Mean values and standard deviations for twelve mice per group)

Diet	A-Smase		N-SMase	
	Mean	SD	Mean	SD
Control	312·5	95·2	78·7	11·1
Psyllium	366·2	90·7	81·0	9·0
High-fat	356·7	81·0	80·1	8·0
High-fat + psyllium	377·9	87·5	85·5	8·3

A, acid; SMase, sphingomyelinase; N, neutral.

* The liver was homogenised and SMase activity determined. For details of diets and procedures, see Tables 1 and 2 and pp. 716–717.

SM metabolism has emerged as a novel signal transduction pathway affecting cell proliferation and apoptosis. The most important lipid messenger derived from SM metabolism is ceramide, which is generated by hydrolysis of SM by SMase or by biosynthesis. Ceramide has been found to inhibit cell proliferation and induce cell differentiation and apoptosis. In general, apoptosis can be accomplished by two major pathways. One pathway is started by the binding of the death receptor, followed by aggregation and activation of caspase 8. The other pathway involves the changes of membrane potential of mitochondria, leading to release of cytochrome *c* and activation of caspase 9. Both caspase 8 and 9 are initiator caspases that will activate caspase 3 downstream, leading to apoptosis. Caspase 3 is the executor of apoptosis, which plays a key role in induction of apoptosis (Reed *et al.* 1996). Under physiological conditions, colonic epithelial cells are rapidly renewing and apoptosis is a critical event in controlling overall colonic tissue homeostasis. The nutritional influence on the apoptotic process has been only addressed recently (Lipkin *et al.* 1999). Our present study showed that the psyllium-containing diet increased caspase activity, indicating the spontaneous apoptotic rate in the colon may be increased by a psyllium-containing diet. The increased caspase 3 activity may be mainly caused by an increased synthesis of the enzyme, as Western blot showed an increased content of caspase 3 precursor in parallel with caspase 3 activity. Caspase 3 is synthesised as a precursor and its activation involves cleavage of a

12 kDa subunit. In the present study, Western blotting failed to identify the cleaved subunit of caspase 3, probably due to the degradation of the subunit during the preparation of the colonic homogenates from the organ. In contrast to psyllium, the high-fat diet decreased both activity and expression of caspase 3 and also significantly reduced the activities of caspase 8 and 9, two initiator caspases. Thus, a high-fat diet is an inhibitor of apoptosis in the colonic mucosa and it can attenuate the pro-apoptotic effect of psyllium.

Although the mechanism by which psyllium and fat affect caspase activity is not clear, the changes in alkaline SMase activity may be a factor responsible for the altered caspase activity. In the intestinal tract, alkaline SMase is the major enzyme responsible for hydrolysis of both endogenous and exogenous SM (Nyberg *et al.* 1997). An increase in alkaline SMase activity by psyllium could elevate the generation of ceramide, which has been shown to induce apoptosis in colonic cells (Veldman *et al.* 1998) and enhance the activities of caspase 3 and 8 in other types of cells (Yoshimura *et al.* 1998; Farina *et al.* 2000; Rodriguez-Lafrasse *et al.* 2001). This hypothesis is supported by the positive correlation of alkaline SMase and caspase 3. The finding is in agreement with one of our previous studies, which showed a positive correlation of alkaline SMase with caspase 3 in rat colon after administration of ursodeoxycholic acid (Cheng *et al.* 1999), a bile salt that has anti-carcinogenic effect in the colon (Earnest *et al.* 1994).

Acid SMase was increased by the high-fat diet and decreased by psyllium in the diet. Acid SMase is a type of lysosomal enzyme and its major function is thought to be hydrolysis of the endocytosed SM. The signalling effect of acid SMase is still a matter of debate (Levade & Jaffrézou, 1999), although in some cell lines such as MCF-7 cells (Chatterjee & Wu, 2001) and murine macrophages (Manthey & Schuchman, 1998; Gomez-Munoz *et al.* 2003) acid SMase was implied to be involved in apoptosis. In human colon cancer tissues, the apoptotic effect of acid SMase has not been reported. In the present study, we found a negative correlation of acid SMase with caspase 3, indicating that acid SMase, by an unknown mechanism, may inhibit colonic apoptosis. It is known that there are different SM pools in the cells that are targeted by different SMase. The final outcome induced by different SMase depends on which SMase is activated, where the ceramide is generated, and what downstream targets are affected by ceramide (Andrieu-Abadie & Levade, 2002; Pettus *et al.* 2002). In addition, the changes in opposite directions of alkaline and acid SMase caused by fat and psyllium implies a cross-regulation of these two SMase. How the regulations are orchestrated is of interest in further investigations.

The mechanisms by which psyllium and a high-fat diet induce the changes in SMase are not clear. Ingestion of psyllium has been found to have multiple effects in the intestinal tract, including generating SCFA (Clausen *et al.* 1991; Edwards & Eastwood, 1995), providing colonic flora with energy and changing the bile acid composition (Trautwein *et al.* 1998). Some of these secondary effects may be involved in increasing the activities of alkaline SMase.

Although the specific factors responsible are unknown, it is clear that the alterations caused by psyllium and fat were not a result of non-specific effects of these agents on lysosomal or brush border enzymes, because the activities of intestinal acid and alkaline phosphatase were not changed in a parallel way. Both acid phosphatase and acid SMase are lysosomal enzymes and both alkaline phosphatase and alkaline SMase are brush border enzymes. Furthermore, the changes in SMase by the diets were also tissue specific and not related to a change in fat absorption, as acid SMase in the liver was not affected by these diets. The alkaline SMase in the mouse liver was not determined, as the enzyme is not expressed in the liver and bile except in human subjects (Duan *et al.* 1996; Cheng *et al.* 2002). Put together, the opposite effects of psyllium-containing and high-fat diets on different types of SMase and caspase indicate a potential biochemical mechanism underlying the differential effects of fat and fibre on tumourigenesis and inflammation in the colon.

Acknowledgements

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