

## Diets enriched with cereal brans or inulin modulate protein kinase C activity and isozyme expression in rat colonic mucosa

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The role of dietary fibres in colon carcinogenesis is controversial. To elucidate the mechanisms by which different dietary fibre sources may affect colonic tumour development, we studied the effects of diets enriched with cereal brans or inulin on protein kinase C (PKC) activity and isozyme expression in rat colon. Male Wistar rats (twelve per group) were fed one of the following AIN-93G-based diets (Reeves *et al.* 1993) for 4 weeks: a non-fibre high-fat diet or one of the four high-fat diets supplemented with either rye, oat or wheat bran or inulin at 100 g/kg diet. The fat concentration (20 g/100 g) and fatty acid composition of the non-fibre high-fat diet was designed to approximate that in a typical Western-type diet. In the proximal colon, rats fed the inulin diet had a significantly higher membrane PKC activity and a higher membrane PKC  $\delta$  level than rats fed the non-fibre diet ( $P < 0.05$ ). In the distal colon, rats fed the inulin and oat bran diets had a higher total PKC activity and a higher membrane PKC  $\beta 2$  level than rats fed the wheat-bran diet. Rats in the non-fibre and wheat-bran groups had the lowest concentrations of luminal diacylglycerol. In conclusion, feeding of wheat bran resulted in low distal PKC activity and expression of PKC  $\beta 2$ , a PKC isozyme related to colonic cell proliferation and increased susceptibility for colon carcinogenesis, which may explain in part the protective effect of wheat bran against tumour development in a number of experimental colon cancer studies. The increase in PKC activity and PKC  $\beta 2$  expression by feeding inulin may be a drawback of inulin as a functional food.

### Cereal brans: Inulin: Protein kinase C isozymes: Colon

Whole-grain intake has been associated with a reduced risk of colon cancer in a number of epidemiological studies (Jacobs *et al.* 1995, 1998). One reason for this association may be that whole grains are important sources of dietary fibres. Fibres are thought to mediate protective effects on colonic epithelium through their fermentation products and faecal bulking capacity. Of the fermentation products, the short-chain fatty acid butyrate is considered to be the key factor by which fibres mediate their protective effect on tumour development. However, a recent study with the azoxymethane colon cancer model in rats demonstrated that large luminal concentrations of butyrate alone are not protective against tumour formation (Zoran *et al.* 1997). Thus, it seems obvious that other mechanisms are involved as well.

Fibres are likely to affect luminal factors, which are able to modify cell signal transduction pathways in colonic mucosal cells. One of these pathways is protein kinase C

(PKC), which constitutes a family of serine and threonine protein kinases with at least eleven isozymes. PKC isozymes differ with respect to tissue distribution, sub-cellular localization and activator requirements, which enable them to mediate a wide range of signals regulating cell growth and differentiation (Nishizuka, 1995). PKC isozymes  $\alpha$ ,  $\beta/\beta 2$ ,  $\delta$ ,  $\epsilon$ , and  $\zeta$  and  $\lambda$  have been reported to be expressed in human and rat colons (Kahl-Rainer *et al.* 1994; Wali *et al.* 1995; Jiang *et al.* 1997a). Of these isozymes, PKC  $\alpha$  and  $\beta$  are so-called conventional, PKC  $\delta$  and  $\epsilon$  are novel, and PKC  $\zeta$  and  $\lambda$  are atypical isoforms. PKC has been implicated in the pathogenesis of colon cancer in many studies. PKC activity has consistently been reduced in colon tumours when compared with the surrounding uninvolved mucosa in colon cancer patients (Guillem *et al.* 1987; Kusunoki *et al.* 1992; Levy *et al.* 1993) as well as in experimental animals (Wali *et al.* 1991; Craven & DeRubertis, 1992). Alterations in isozyme

**Abbreviations:** DAG, diacylglycerol; PKC, protein kinase C.

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expression have also been reported, so that most studies have shown reduced protein expression of one or more PKC isozymes in colon tumours when compared with the normal mucosa (Kahl-Rainer *et al.* 1994; Pongracz *et al.* 1995; Wali *et al.* 1995). PKC  $\beta/\beta 2$  protein levels, however, have been unchanged or increased in tumours (Craven & DeRubertis, 1992; Davidson *et al.* 1994; Kahl-Rainer *et al.* 1994; Wali *et al.* 1995). Studies utilising experimental colon cancer models have demonstrated that alterations in PKC activity and isozyme expression occur before tumour formation and involve a persistent activation and translocation and subsequent down regulation of the enzyme (Baum *et al.* 1990; Wali *et al.* 1991; Craven & DeRubertis, 1992; Jiang *et al.* 1997b). These results suggest that PKC may have an active role already at the early stages of colonic malignant transformation.

One of the luminal factors that may alter PKC activity and isozyme expression is diacylglycerol (DAG). DAG is a physiological activator for conventional and novel PKC isozymes and it has been shown to be present in faeces in appreciable amounts, probably due to the action of colonic bacteria on undigested dietary residues (Morotomi *et al.* 1990). Luminal DAG is capable of entering colonic epithelial cells (Morotomi *et al.* 1991), and thus it may contribute to mucosal PKC activity. There is some evidence that certain dietary fibre sources may alter the production and composition of DAG (Reddy *et al.* 1994; Pickering *et al.* 1995).

The purpose of the present study was to determine whether different grain brans are capable of modifying colonic PKC activity and isozyme levels, and if so, whether it is due to changes in luminal DAG concentration. The brans chosen were wheat, oat and rye bran. Wheat bran contains poorly-fermentable fibre and has been consistently shown to prevent colonic tumour formation in experimental colon cancer models (McIntry *et al.* 1993; Zoran *et al.* 1997). Oat-bran fibre is readily fermented and has rather promoted than inhibited tumour development (Jacobs & Lupton, 1986; McIntry *et al.* 1993; Zoran *et al.* 1997). The composition of rye bran is similar to that of wheat bran, and rye supplementation has been demonstrated to decrease colonic tumour development in carcinogen-treated rats (Davies *et al.* 1999). In addition to the fibre component, cereal brans contain a number of bioactive compounds, such as phytate and lignans, which have been suggested to protect against colon cancer (Adlercreutz & Mazur, 1997; Jenab & Thompson, 1998). It is possible that these compounds could affect PKC expression and activity. An inulin-supplemented group was also included in the present study because we were interested in comparing the effects of this highly-fermentable fibre with those of grain brans. Inulin, a chicory (*Cichorium intybus*) fructan, has attracted considerable attention in recent years as a potential health-promoting food, i.e. a functional food (Gibson & Roberfroid, 1995). With respect to colon carcinogenesis, inulin has been found to reduce preneoplastic aberrant crypt foci formation in rat colon (Rowland *et al.* 1998). The effects of the different fibre sources on PKC were primarily compared with those of a non-fibre high-fat control diet, but also with those of wheat bran (positive control) because of its established protective role in experimental colon carcinogenesis.

## Materials and methods

### Animals and diets

The animal use protocol for the present experiment was approved by the Laboratory Animal Ethics Committee of the Faculty of Agriculture and Forestry, University of Helsinki. Sixty male Wistar rats (Harlan Co., Horst, The Netherlands), initially weighing 116–144 g, were used. They were maintained in plastic cages (three rats per cage) in a room with a controlled temperature (20–22°C) and a 12 h light–dark cycle for the duration of the study. Wood-chip bedding of aspen (*Populus tremula*) was used, and therefore some consumption of bedding, and thus wood fibre, might have occurred. After a 1-week acclimation period, the rats were divided into five dietary treatment groups, which were equal in weight (twelve rats per diet). They were fed on their respective diets for 4 weeks, and thereafter they were killed during a 2-week period so that the same number of rats from each dietary group was killed on each day. Rats had free access to diet and tap water throughout the study. Their body weights were recorded weekly. At the end of the study, each rat was kept in a metabolic cage for a 48 h period to obtain food consumption and faecal and urine output data.

The semi-synthetic experimental diets were modified AIN-93G diets (Reeves *et al.* 1993) and were composed so that they were similar with respect to protein, fat, vitamin and mineral content on an energy basis. The experimental diets included a non-fibre high-fat diet and four high-fat diets supplemented with one of the following fibre sources: rye bran, oat bran, wheat bran or inulin (Table 1). The non-fibre diet was designed to approximate to an average human Western-type diet, so that its fat content was high, i.e. 40 % energy, and saturated:monounsaturated:polyunsaturated fatty acids was 3:2:1 in relation to energy provided by fat. The fibre-supplemented diets were prepared by diluting the non-fibre high-fat diet with the addition of fibre source at 100 g/kg diet. Due to differences in fibre concentration in the brans, the amount of fibre was 34 g/kg

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

Ingredient	Diet		
	Non-fibre	Cereal bran	Inulin
Casein	236.2	212.6	212.6
Dextrose	479.0	431.1	431.1
Butter	148.9	134.0	134.0
Sunflower-seed oil	13.3	12.0	12.0
Rapeseed oil	62.2	56.0	56.0
Mineral mixture*	41.6	37.4	37.4
Vitamin mixture†	11.8	10.6	10.6
L-Cystine	3.6	3.2	3.2
Choline chloride	3.6	3.2	3.2
Tertiary butylhydroxyquinone	0.014	0.014	0.014
Cereal bran‡	–	100.0	–
Inulin§	–	–	100.0

\* AIN-93 mineral mixture (Harlan Teklad, Madison, WI, USA).

† AIN-93 vitamin mixture (Harlan Teklad, Madison, WI, USA).

‡ Wheat-, oat-, and rye-bran-enriched diets were included in the study. Wheat bran contained 41 % insoluble and 1.8 % soluble fibre; oat bran contained 12 % insoluble and 6.2 % soluble fibre; rye bran contained 30 % insoluble and 4.3 % soluble fibre.

§ Raftiline HP®, 99.5 % inulin, solid; Orafit, Tienen, Belgium.

in the rye-bran diet, 18 g/kg in the oat-bran diet and 43 g/kg in the wheat-bran diet. In order to achieve the same level of fibre as in the wheat-bran diet, oat bran should have been added to the basal diet at 233 g/kg. This level of addition would have led to substantial differences in protein, lipid and carbohydrate composition of the diets, and we chose, therefore, to keep the differences that also occur when these cereal brans are used as fibre sources in human diets. The fibre levels in the bran diets correspond to 10–23 g fibre/d in human subjects, which can be considered a moderate fibre intake in light of the currently-recommended 20 g/d. The fibre concentration in the inulin diet was 100 g/kg diet, which is the level of inulin that has been described to have beneficial effects on human health (Gibson & Roberfroid, 1995).

#### *Tissue preparation and protein extraction*

At the end of the feeding period the rats were killed by CO<sub>2</sub> asphyxiation. Their colons were removed and cut open longitudinally. The contents of the distal colon were collected, snap-frozen in liquid N<sub>2</sub> and stored at –80°C until analysed for DAG concentration. The colons were flushed clean with ice-cold PBS, divided into proximal and distal segments of equal length and the mucosa was scraped off with a microscope slide. Cytosolic and membrane proteins from the mucosal sample were extracted as described previously (Pajari *et al.* 1998). Briefly, homogenised mucosa was ultracentrifuged at 100 000 g for 1 h, and the supernatant fraction was collected and used as the cytosolic fraction. The pellet was resuspended in the extraction buffer containing 0.2 % (v/v) Triton X-100, incubated for 20 min and ultracentrifuged at 100 000 g for 1 h. The resulting supernatant fraction contained the membrane fraction. Cytosolic (2 ml) and membrane (2.75 ml) fractions were further purified by DEAE–Sephacel chromatography and used for PKC activity measurement (Pajari *et al.* 1998). The protein concentration of the crude and DEAE-purified fractions was measured using a protein assay reagent (Bradford; Bio-Rad, Hercules, CA, USA) with bovine serum albumin as a standard.

#### *Immunoblotting*

For immunoblotting analysis, 2 ml of the crude cytosolic and membrane extracts were concentrated to 1/20 volume with Centrex UF-2 concentrators (Schleicher & Schuell, Dassel, Germany). The retentive was mixed with an equal volume of SDS-sample buffer, boiled for 5 min and stored at –80°C until use. One pooled rat-brain homogenate, obtained from three Wistar rats and containing both cytosolic and membrane proteins, was prepared essentially in the same way as described for the colon samples. Rat-brain homogenate was used as a positive control for PKC antibody specificity and as an internal control to avoid inter-assay variation. Colonic mucosa samples (30–100 µg) and a constant amount of rat-brain homogenate (1–5 µg) were subjected to 10 % SDS–PAGE (Laemmli, 1970) and then electroblotted (Towbin *et al.* 1979) onto polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA, USA) at 210 mA for 3 h. Transfer of proteins to the

membranes was confirmed by staining gels with 0.1 % (w/v) Coomassie Brilliant Blue R-250 in fixative (methanol (40 %, v/v), acetic acid (10 %, v/v)). The membranes were further processed by the method of Sheng & Schuster (1992). The membranes were blocked with 10 g non-fat dry milk/l PBS at 4°C overnight, washed with 5 g bovine serum albumin/l (Sigma, St Louis, MO, USA) in PBS, and incubated with antibodies for PKC isozymes in 10 g bovine serum albumin/l in PBS. PKC bands were visualised by colorimetric staining of blots with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium substrate mix (Bio-Rad). Blots were scanned on a Sharp JX325 Scanner (Pharmacia Amersham Biotech, Amersham Bucks., UK) and the scanning images were analysed with ImageMaster<sup>®</sup> 1D Software, version 2.0 (Pharmacia Biotech, Uppsala, Sweden). The intensity of each colon sample was normalised to the intensity of rat-brain homogenate derived from the same gel. The results are expressed as sample band intensity (optical density of the specific PKC band multiplied by band area) divided by rat-brain band intensity. In preliminary experiments a range of protein concentrations for each isozyme was loaded onto the gels to ensure that the colorimetric signal was quantitatively detectable. Representative immunoblots with respect to the quantitative detection of protein concentrations and each PKC isozyme in the colon and brain are shown in Fig. 1.

We were able to detect PKC  $\alpha$ ,  $\beta$ 2,  $\delta$ , and  $\zeta$  in rat colonic mucosa, whereas PKC  $\epsilon$ ,  $\lambda$ ,  $\mu$ , and  $\theta$  gave no accurately measurable signal. Dilution of individual primary antibody and alkaline phosphatase-conjugated secondary antibody was optimised for each PKC isozyme. The antibodies for the PKC  $\alpha$  and  $\zeta$  isozymes react, to a lesser extent, with  $\beta$  and  $\lambda(\iota)$  isozymes respectively. PKC  $\beta$ 2 and  $\delta$  antibodies are non-cross-reactive with other PKC isoforms. The blocking peptide of PKC  $\zeta/\lambda$  was used to control the specificity of the antiserum. For this blocking test, a ten-fold (by weight) excess of peptide antigen was incubated with PKC  $\zeta/\lambda$  antibody in 1 % (w/v) BSA in PBS overnight at 4°C. Subsequent washes, addition of antibodies and colorimetric staining were performed as described for the samples.

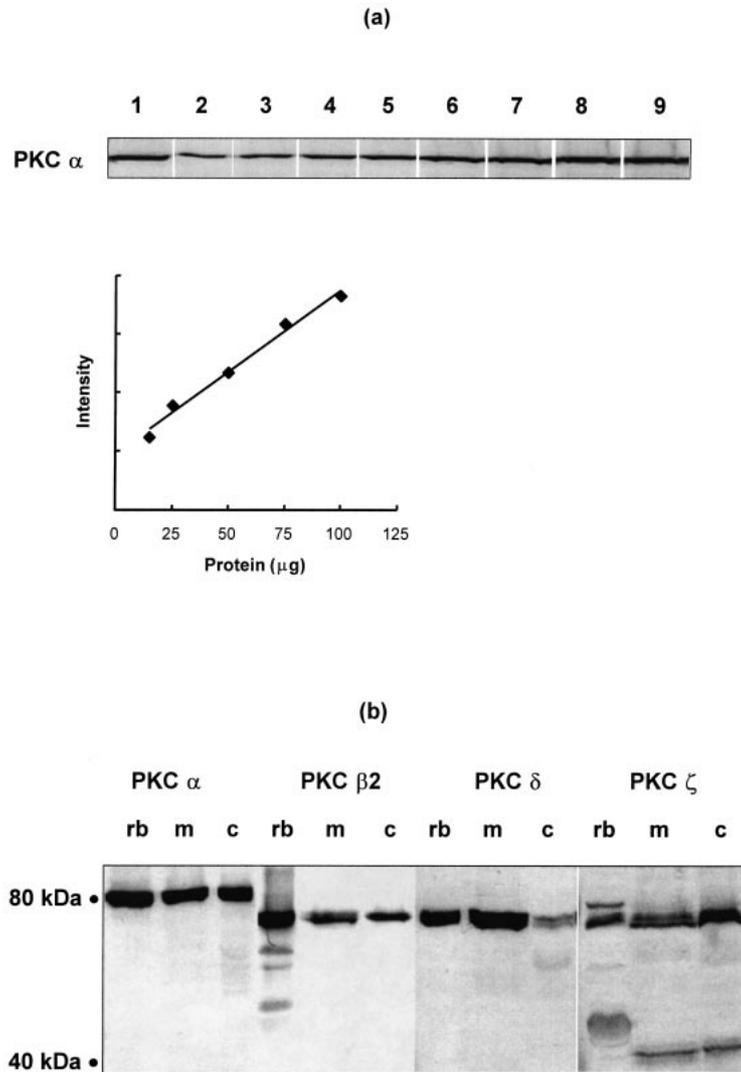
Monoclonal mouse antibodies for PKC  $\alpha$  and  $\delta$  were purchased from Transduction Laboratories (Lexington, KY, USA) and alkaline phosphatase-conjugated anti-mouse secondary antibody were obtained from Bio-Rad Laboratories. Polyclonal rabbit anti-PKC  $\beta$ 2 and  $\zeta/\lambda$ , blocking peptide for PKC  $\zeta/\lambda$ , and alkaline phosphatase-conjugated anti-rabbit secondary antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

#### *Luminal diacylglycerol*

DAG concentration from the distal colonic contents was analysed essentially in the same way as described previously (Pajari *et al.* 1998) using a commercial kit (Amersham International plc, Amersham, Bucks., UK).

#### *Statistical analyses*

Results are presented as means and standard deviations.



**Fig. 1.** (a) Representative blot showing quantitative detection of protein kinase C (PKC)  $\alpha$  in rate colon mucosal sample by immunoblotting. Lane 1 rat-brain homogenate; lanes 2–9 5, 10, 15, 25, 50, 75, 100 and 125  $\mu\text{g}$  protein in colon mucosal sample. Sample intensity was linear from 15 to 100  $\mu\text{g}$  ( $r^2$  0.98). Linearity for PKC  $\beta$ 2,  $\delta$ , and  $\zeta$  was checked in the same way (not shown). (b) Representative immunoblots for PKC  $\alpha$ ,  $\beta$ 2,  $\delta$ , and  $\zeta$  in rat brain (rb), and cytosolic (c) and membrane (m) fractions of rat colon mucosal sample.

Means are given for twelve rats per diet group, except for the following: luminal DAG concentration is given for ten to twelve rats per group because some rats had nearly empty colons and thus no faecal contents were available for DAG analysis; PKC  $\alpha$ ,  $\delta$ , and  $\zeta/\lambda$  levels are given for ten to twelve rats because values with outlying studentised residuals were considered outliers and were excluded; PKC  $\beta$ 2 levels were determined last and due to limited sample material analyses were done from nine rats per group. Data were analysed by one-way ANOVA and Tukey's *post hoc*-test, with differences of  $P < 0.05$  considered to be significant. The correlation data were analysed by a linear regression analysis. The SYSTAT for Windows, version 7.0 (SPSS Inc., Chigaco, IL, USA) was used for all the statistical analyses.

## Results

### *Food and energy intake, body weight, tissue weight and length, and faecal weight*

Food intakes and final body weights did not differ among the dietary groups. The mean values of the final body weights (g) in different dietary groups were as follows: the non-fibre group 348, the wheat-bran group 351, the rye-bran group 346, the oat-bran group 347, and the inulin group 349 (pooled SD 25, ANOVA  $P = 0.158$ ). Rats fed the wheat-bran and inulin diets had a greater ( $P < 0.01$ ) faecal output than rats fed the non-fibre diet (Table 2). The weights of caecal mucosa and contents were greater ( $P < 0.0001$ ) in rats fed the inulin diet than in rats fed the other diets (Table 2). There were no

**Table 2.** Caecal weight (g) and faecal excretion (g/48 h) of rats fed on diets enriched with cereal brans or inulin\*

(Mean values and standard deviations for twelve rats)

Diet†	Caecum with contents		Caecal mucosa		Faecal excretion	
	Mean	SD	Mean	SD	Mean	SD
Non-fibre	1.85 <sup>a</sup>	0.45	0.14 <sup>a</sup>	0.03	3.1 <sup>a</sup>	1.0
Wheat bran	2.35 <sup>a</sup>	0.41	0.17 <sup>a</sup>	0.04	4.7 <sup>b</sup>	1.5
Rye bran	2.26 <sup>a</sup>	0.54	0.14 <sup>a</sup>	0.03	4.0 <sup>ab</sup>	1.2
Oat bran	2.19 <sup>a</sup>	0.47	0.17 <sup>a</sup>	0.04	2.9 <sup>a</sup>	0.8
Inulin	4.89 <sup>b</sup>	1.02	0.31 <sup>b</sup>	0.09	4.8 <sup>b</sup>	1.0

<sup>a,b</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of procedures, see p. 636.

† For details of composition, see Table 1.

differences between the groups in colon length or mucosal weight (data not shown).

### PKC isozyme expression

We were able to obtain quantitative signals for PKC  $\alpha$ ,  $\beta_2$ ,  $\delta$ , and  $\zeta/\lambda$  in rat colonic mucosa. Rat colon samples and rat-brain homogenate gave a single band for PKC  $\alpha$  and  $\beta_2$  at approximately 80 and 74 kDa respectively. PKC  $\delta$  was detected as a 73 kDa band in brain and as a 72 and 69 kDa doublet in colon samples. PKC  $\delta$  was under the detection limit in cytosolic samples from the proximal colon. PKC  $\zeta/\lambda$  gave three bands at 75, 70 and 50 kDa in brain, four bands at 75, 70, 48 and 45 kDa in proximal colon samples, and four bands at 75, 70, 43 and 40 kDa in distal colon samples, all of which were eliminated when the blocking peptide of PKC  $\zeta/\lambda$  was pre-incubated with PKC  $\zeta/\lambda$  antibody.

Feeding diets enriched with different fibre sources resulted in significant but variable differences in colonic PKC isozyme expression (Tables 3 and 4). In the proximal colon, rats fed the inulin diet had a higher ( $P < 0.05$ ) level of membrane PKC  $\delta$  than those fed the non-fibre diet. Other changes occurred in the cytosolic fraction so that rats fed the oat-bran diet had a significantly ( $P = 0.022$ ) lower

level of cytosolic PKC  $\alpha$  than rats fed the non-fibre diet (Table 3), and rats fed the inulin diet had a higher ( $P < 0.05$ ) level of cytosolic PKC  $\zeta/\lambda$  than rats in the non-fibre and wheat-bran groups (Table 3). The differences in PKC  $\zeta/\lambda$  expression could be seen in both the total PKC  $\zeta/\lambda$  and 70 and 75 kDa fractions. There were no differences in PKC  $\beta_2$  levels among the diet groups (Table 3).

In the distal colon membrane PKC  $\beta_2$  levels were significantly ( $P < 0.05$ ) higher in rats fed the inulin and oat-bran diets than in those fed the wheat-bran diet (Table 4). Rats fed the inulin diet also had the highest PKC  $\beta_2$  level in the cytosolic fraction and they differed significantly from rats fed the non-fibre ( $P = 0.003$ ) and wheat-bran ( $P = 0.041$ ) diets. Cytosolic PKC  $\beta_2$  expression in the rye-bran group was similar to that in the inulin group, and it differed significantly ( $P = 0.031$ ) from that in the non-fibre group. The inulin diet resulted in the highest cytosolic PKC  $\delta$  and  $\zeta/\lambda$  expression, and the oat-bran diet the lowest levels of these isozymes (Table 4). PKC  $\alpha$  expression was not affected by the diets (Table 4).

Since many PKC isozymes translocate to the membrane on activation, membrane:cytosol levels for each isozyme was calculated for all the dietary groups. The diets used in the present study had no significant effect on membrane:cytosol distribution of isozymes in any subsides of the colon (data not shown).

### PKC activity and luminal diacylglycerol concentration

In the proximal colon PKC activity in rats fed on the cereal-bran diets did not differ significantly from that in the non-fibre group (Table 5). However, rats fed the inulin diet had significantly ( $P < 0.05$ ) higher membrane PKC activity than rats in the other dietary groups, except in the oat-bran group. The membrane:cytosol level in the inulin group differed significantly ( $P = 0.019$ ) from that in the non-fibre group. In the distal colon, rats fed the oat-bran and inulin diets had a significantly ( $P < 0.05$ ) higher total PKC activity than rats in the wheat-bran group (Table 5). Membrane PKC activity in the proximal colon was significantly correlated with membrane PKC  $\alpha$  ( $r = 0.37$ ,  $P = 0.015$ ) and PKC  $\delta$  ( $r = 0.50$ ,  $P = 0.002$ ) levels. In the distal

**Table 3.** Protein kinase C (PKC) isozyme expression (relative intensity) in proximal colonic mucosa of rats fed on diets enriched with cereal brans or inulin\*†

(Mean values and standard deviations for nine to twelve rats)

Diet‡	Membrane PKC $\delta$		Cytosolic PKC $\alpha$		Cytosolic PKC $\zeta/\lambda$	
	Mean	SD	Mean	SD	Mean	SD
Non-fibre (NF)	0.25 <sup>a</sup>	0.10	0.39 <sup>a</sup>	0.18	1.01 <sup>a</sup>	0.23
Wheat bran (WB)	0.40 <sup>ab</sup>	0.20	0.28 <sup>ab</sup>	0.08	1.04 <sup>a</sup>	0.27
Rye bran (RB)	0.35 <sup>ab</sup>	0.11	0.30 <sup>ab</sup>	0.09	1.25 <sup>a</sup>	0.42
Oat bran (OB)	0.28 <sup>a</sup>	0.14	0.23 <sup>b</sup>	0.06	0.99 <sup>a</sup>	0.27
Inulin (INU)	0.55 <sup>b</sup>	0.24	0.35 <sup>ab</sup>	0.13	1.42 <sup>b</sup>	0.49

<sup>a,b</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* There were no diet-induced changes in the expression of the following PKC isozymes: membrane PKC  $\alpha$  (mean): NF 0.10, WB 0.08, RB 0.07, OB 0.10, INU 0.09 (pooled SD 0.04, ANOVA  $P = 0.911$ ); membrane PKC  $\beta_2$ : NF 0.14, WB 0.13, RB 0.12, OB 0.16, INU 0.11 (pooled SD 0.07, ANOVA  $P = 0.829$ ); cytosolic PKC  $\beta_2$ : NF 0.47, WB 0.50, RB 0.54, OB 0.49, INU 0.39 (pooled SD 0.22, ANOVA  $P = 0.833$ ); membrane PKC  $\zeta/\lambda$ : NF 0.90, WB 0.59, RB 0.58, OB 0.55, INU 0.80 (pooled SD 0.39, ANOVA  $P = 0.923$ ).

† For details of procedures, see p. 636.

‡ For details of composition, see Table 1.

**Table 4.** Protein kinase C (PKC) isozyme expression (relative intensity) in distal colonic mucosa of rats fed on diets enriched with cereal brans or inulin\*†

Diet‡	(Mean values and standard deviations for nine to twelve rats)							
	Membrane PKC $\beta$ 2		Cytosolic PKC $\beta$ 2		Cytosolic PKC $\delta$		Cytosolic PKC $\zeta/\lambda$	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Non-fibre (NF)	0.12 <sup>ab</sup>	0.04	0.30 <sup>a</sup>	0.17	0.86 <sup>ab</sup>	0.83	0.81 <sup>ab</sup>	0.27
Wheat bran (WB)	0.09 <sup>a</sup>	0.03	0.39 <sup>ab</sup>	0.14	0.91 <sup>ab</sup>	0.58	0.89 <sup>ab</sup>	0.49
Rye bran (RB)	0.15 <sup>ab</sup>	0.07	0.56 <sup>bc</sup>	0.24	0.87 <sup>ab</sup>	0.65	0.68 <sup>ab</sup>	0.24
Oat bran (OB)	0.17 <sup>b</sup>	0.07	0.49 <sup>ab</sup>	0.14	0.56 <sup>a</sup>	0.29	0.52 <sup>a</sup>	0.27
Inulin (INU)	0.17 <sup>b</sup>	0.05	0.63 <sup>c</sup>	0.14	1.47 <sup>b</sup>	1.01	0.94 <sup>b</sup>	0.41

<sup>a,b</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* There were no diet-induced changes in the expression of the following PKC isozymes: membrane PKC  $\alpha$ : NF 0.05, WB 0.06, RB 0.05, OB 0.05, INU 0.08 (pooled SD 0.05, ANOVA  $P = 0.927$ ); cytosolic PKC  $\alpha$ : NF 0.28, WB 0.25, RB 0.23, OB 0.22, INU 0.30 (pooled SD 0.14, ANOVA  $P = 0.427$ ); membrane PKC  $\delta$ : NF 2.98, WB 2.42, RB 2.51, OB 2.77, INU 3.14 (pooled SD 1.46, ANOVA  $P = 0.635$ ); membrane PKC  $\zeta/\lambda$ : NF 0.89, WB 0.90, RB 0.83, OB 0.90, INU 0.96 (pooled SD 0.39, ANOVA  $P = 0.923$ ).

† For details of procedures, see p. 636.

‡ For details of composition, see Table 1.

**Table 5.** Protein kinase C (PKC) activity (pmol/min per mg protein) in proximal and distal colonic mucosa of rats fed on diets enriched with cereal brans or inulin\*

Diet†	(Mean values and standard deviations for twelve rats)					
	Membrane		Membrane+cytosol		Membrane:cytosol	
	Mean	SD	Mean	SD	Mean	SD
Proximal colon						
Non-fibre	3144 <sup>a</sup>	1151	5095 <sup>ab</sup>	1353	1.7 <sup>a</sup>	0.7
Wheat bran	3544 <sup>a</sup>	1109	5222 <sup>ab</sup>	944	2.4 <sup>ab</sup>	1.3
Rye bran	3302 <sup>a</sup>	1358	4990 <sup>a</sup>	1312	2.2 <sup>ab</sup>	1.3
Oat bran	4054 <sup>ab</sup>	1400	5870 <sup>ab</sup>	1568	2.5 <sup>ab</sup>	1.2
Inulin	5064 <sup>b</sup>	1355	6608 <sup>b</sup>	1344	3.6 <sup>b</sup>	1.8
Distal colon						
Non-fibre	2424	914	4341 <sup>ab</sup>	986	1.4	0.8
Wheat bran	2107	518	3869 <sup>a</sup>	578	1.4	0.9
Rye bran	2223	718	3923 <sup>a</sup>	814	1.6	1.1
Oat bran	2771	1029	4784 <sup>b</sup>	1190	1.8	1.2
Inulin	2773	778	4612 <sup>b</sup>	783	1.8	1.0

<sup>a,b</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of procedures, see p. 636.

† For details of composition, see Table 1.

colon, membrane activity was significantly correlated with membrane PKC  $\beta$ 2 ( $r$  0.44,  $P = 0.004$ ) and PKC  $\zeta$  ( $r$  0.38,  $P = 0.004$ ) levels. Cytosolic PKC activity was not correlated with any isozyme.

Rats fed the non-fibre and wheat-bran diets had the lowest luminal DAG concentrations and they differed

**Table 6.** Diacylglycerol concentration (ng/wet weight faeces) in distal colonic contents of rats fed on diets enriched with cereal brans or inulin\*

Diet	(Mean values and standard deviations for ten to twelve rats)	
	Diacylglycerol	
	Mean	SD
Non-fibre	136 <sup>a</sup>	73
Wheat bran	141 <sup>a</sup>	63
Rye bran	242 <sup>b</sup>	76
Oat bran	210 <sup>ab</sup>	87
Inulin	183 <sup>ab</sup>	103

<sup>a,b</sup>Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* For details of procedures, see p. 636.

† For details of composition, see Table 1.

significantly ( $P < 0.05$ ) from that of the rats fed the rye-bran diet (Table 6). DAG concentrations in the inulin and oat-bran groups were between these two extremes. Luminal DAG was significantly correlated with distal cytosolic PKC  $\beta$ 2 levels ( $r$  0.41,  $P = 0.019$ ), but not with other PKC isozymes or PKC activity.

## Discussion

The present study describes the effects of different dietary fibre sources, i.e. wheat bran, rye bran, oat bran and inulin on colonic PKC activity and isozyme protein levels. PKC isozymes play an important role in cell signal transduction, and thereby contribute to cell proliferation, differentiation, and apoptosis. The present study demonstrated that effects of different fibre sources on colonic PKC activity and isozyme expression vary depending on the site of the colon. Since colon tumours are known to be more common in the distal site in high-incidence Western countries (Bufill, 1990), the changes seen in distal PKC may be the most important when considering the effects of diet on colon carcinogenesis. In this respect, rats fed the oat-bran and

inulin diets showed an increase in membrane PKC  $\beta$ 2 protein levels as well as PKC activity in the distal colonic mucosa when compared with the rats in the wheat-bran group. There was also a significant correlation between membrane PKC  $\beta$ 2 and PKC activity in the distal colon. Rats fed the rye-bran diet had an elevated cytosolic PKC  $\beta$ 2 level but no increase in their membrane or total PKC activity, which were as low as those in rats fed the wheat-bran diet.

Several lines of evidence suggest that PKC  $\beta$ 2 mediates colonic cell proliferation, and that elevated PKC  $\beta$ 2 expression and activity enhance colon carcinogenesis. In carcinogen-injected animals both PKC  $\beta$ 2 expression and membrane-particulate association were increased in tumours relative to the uninvolved surrounding mucosa (Wali *et al.* 1995), suggesting that membrane association of PKC  $\beta$ 2 may be related to the growth advantage of tumour cells. In a colon cancer cell line overexpression of PKC  $\beta$ 2 led to blocked differentiation, increased growth rate in athymic mice and restoration of the response for the basic fibroblast growth factor (Sauma & Friedman 1996; Sauma *et al.* 1996). Most importantly, a recent *in vivo* study showed that transgenic mice overexpressing PKC  $\beta$ 2 exhibited hyperproliferation in their colonic epithelium as well as an increased susceptibility to azoxymethane-induced aberrant crypt foci, preneoplastic lesions in the colon (Murray *et al.* 1999).

The PKC  $\beta$ 2 data in our study are in agreement with the fact that wheat and oat brans have been shown to have different effects on colonic tumour formation in experimental colon cancer models. Wheat bran has protected against tumour development, whereas readily-fermentable fibres, such as oat bran, pectin and guar gum, have rather promoted than inhibited tumour development in rats (Jacobs & Lupton, 1986; McIntry *et al.* 1993; Zoran *et al.* 1997). The effect of inulin on colon carcinogenesis is yet to be established, although inulin has been found to reduce preneoplastic aberrant crypt foci formation in rat colon when added either to a basal AIN-76 diet (Reddy *et al.* 1997) or a high-fat maize-oil diet (Rowland *et al.* 1998). However, the low fat concentration and the presence of cellulose in the AIN-76 diet as well as the high *n*-6 fatty acid content of the maize-oil diet may not optimally reflect a human high-risk diet that is considered to be high in saturated fat and low in fibre.

In the proximal colon the effects of different fibre sources on PKC activity were nearly identical with those seen in the distal colon. Ingestion of the inulin diet and, to a lesser extent the oat-bran diet, resulted in an increased membrane and total PKC activity when compared with the other diets. However, the effects of the diets on isozyme expression differed from those in the distal colon. Rats fed the inulin diet had a clearly higher PKC  $\delta$  level in the membrane fraction than rats fed the non-fibre diet. This result together with the significant correlation between PKC activity and PKC  $\delta$  levels indicate activation of this particular isozyme in the inulin group. Unlike PKC  $\beta$ 2, PKC  $\delta$  protein levels have been down regulated in colonic tumours compared with the surrounding normal mucosa (Kahl-Rainer *et al.* 1994; Wali *et al.* 1995). Studies with experimental colon cancer models suggest that this down

regulation is a consequence of a persistent activation and translocation of the enzyme, which occur before tumour formation (Baum *et al.* 1990; Wali *et al.* 1991; Craven & DeRubertis, 1992; Jiang *et al.* 1997b). Thus, the elevated membrane PKC activity in rats fed the inulin diet may indicate that these animals are prone to down regulation of PKC  $\delta$ . This factor is important because PKC  $\delta$  has been shown to suppress growth and reverse the transformed phenotype of the cells in an *in vitro* model of colon carcinogenesis (Perletti *et al.* 1999).

Other dietary-induced changes in PKC expression occurred in the levels of cytosolic PKC  $\alpha$  and  $\zeta/\lambda$  in the proximal colon and in the levels of cytosolic PKC  $\delta$  and  $\zeta/\lambda$  in the distal colon. PKC  $\alpha$  levels were lower in rats fed the cereal-bran diets than in those fed the non-fibre diet. With respect to cytosolic PKC  $\delta$  and  $\zeta/\lambda$ , rats fed the oat-bran diet had the lowest levels and rats fed the inulin diet had the highest levels, while the values for the other dietary groups were between these two extremes. Studies with colon carcinoma cells have suggested that PKC  $\alpha$  mediates cell differentiation and growth arrest (Frey *et al.* 1997; Scaglione-Sewell *et al.* 1998), whereas atypical PKC  $\zeta$  and  $\lambda$  have been implicated in mediating growth, differentiation and maturation in several cell types (Dominguez *et al.* 1992; Berra *et al.* 1993; Liu *et al.* 1998). Since no correlation between PKC activity and cytosolic PKC  $\delta$  and  $\zeta$  levels in the distal colon nor PKC activity and cytosolic PKC  $\alpha$  and  $\zeta$  levels in the proximal colon could be found, the importance of the changes in cytosolic PKC isozyme levels for these cellular functions remains uncertain.

Modulation of luminal DAG production is considered to be a mechanism by which diet may affect mucosal PKC activity in the colon (Morotomi *et al.* 1991). Indeed, dietary fibres, cellulose and pectin, can alter composition and excretion of faecal DAG in rats (Pickering *et al.* 1995), and a supplement of 15 g wheat bran/d for 8 weeks reduces the concentration of faecal DAG in women (Reddy *et al.* 1994). In the present study, DAG concentration in the distal colonic contents was lowest in rats fed the non-fibre and wheat-bran diets, whereas rats fed the rye-bran, oat-bran and inulin diets had elevated luminal DAG levels. Thus, high luminal DAG concentrations might have contributed to the increased PKC activity in the oat-bran- and inulin-fed rats. However, there is a discrepancy between the high DAG level and low PKC activity in the rye-bran group, which argues against the major role of luminal DAG in regulating mucosal PKC activity. Overall, the relationship between luminal DAG and mucosal PKC seems to be complex, and changes in DAG production can explain only partly the effects of the fibre sources on colonic PKC activity in the present study. Cereal brans do contain many bioactive compounds, such as phytates and phyto-oestrogens, which might also affect colonic signal transduction.

In conclusion, the present study demonstrated that diets enriched with different fibre sources could have very different effects on colonic PKC activity and isozyme expression. Specifically, feeding of the wheat-bran-enriched diet resulted in a low distal PKC activity and expression of PKC  $\beta$ 2, a PKC isozyme related to colonic cell proliferation and increased susceptibility for colon

carcinogenesis. The favourable effects of wheat bran on PKC activity and isozyme expression may explain in part the protective effect of wheat bran against tumour development in a number of experimental colon cancer studies. However, ingestion of the inulin-enriched diet resulted in an increased PKC activity and PKC  $\beta_2$  level in the distal colonic mucosa, indicating that this highly-fermentable fructose polymer may enhance colon carcinogenesis. This possibility is supported by the result of our latest study which demonstrated that inulin at a level of 25 g/kg diet promoted intestinal tumour development in Min mice (Mutanen *et al.* 2000).

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