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Phospholipid fatty acid composition and protein kinase C activity in the large intestine of rats fed on butter and coconut-oil diets

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Protein kinase C (PKC) has been proposed to play an important role in the aetiology of colon cancer. Therefore, we investigated whether the amount and type of saturated fat could affect colonic PKC activity by modifying either mucosal phospholipid fatty acid composition or faecal diacylglycerol production. Male Wistar rats (*n* 13 per group) were fed on diets containing butter or coconut oil at energy levels of 10 % and 43 % for 4 weeks. The control group received a low-fat diet providing 10 % of energy from sunflowerseed oil. PKC activity was higher in the distal than the proximal colon but the quantity or type of fat did not alter PKC activity in either region of the colon. Saturated fats caused moderate changes in the fatty acid composition of caecal phospholipids, which were more obvious in the phosphatidylethanolamine than in the phosphatidylcholine fraction. A significant correlation was found between fatty acid composition of phosphatidylcholine and membrane PKC activity. In particular, there was a positive correlation between the proportion of saturated 14:0 and 18:0 and increased PKC activity while unsaturated 18:2*n*-6, 20:4*n*-6 and 16:1*n*-7 were inversely correlated with PKC activity. No relationship was found between phosphatidylethanolamine fatty acids and PKC activity. Concentration of faecal diacylglycerol was not affected by the diet. Overall the data suggest that diets high in saturated fat may not alter colonic PKC activity to a significant extent.

Coconut oil: Phospholipids: Protein kinase C: Large intestine

Data from epidemiological studies (Potter *et al*. 1993) as well as experimental cancer models in animals (Reddy, 1992) suggest that consumption of diets high in fat, saturated fat in particular, is associated with increased risk of colon cancer. Because the evidence has been partly controversial, studies on the underlying mechanisms between fat and colon cancer are warranted.

One possible mechanism may be activation of different protein kinase C (PKC) isoenzymes which are involved in a variety of cellular functions, including cell growth, tumour promotion, differentiation, and cell death (Nishizuka, 1984, 1995; Blobe *et al*. 1994; Livneh & Fishman, 1997). PKC consists of a family of serine/threonine protein kinases with at least eleven isoenzymes which differ with respect to tissue distribution, subcellular localization, and activator requirements (Nishizuka, 1995). PKC isoforms α, β/β_2, δ, ε, and ζ have been reported to be expressed in human as well as rat colons (Davidson *et al*. 1994; Kahl-Rainer *et al*. 1994; Wali *et al*. 1995). PKC-α and β_2 are so-called conventional isoforms which are regulated by Ca, diacylglycerol (DAG), and phosphatidylserine. PKC-δ and -ε are novel isoforms which are Ca independent but require DAG and phosphatidylserine for activation. PKC-ζ, and -λ are atypical isoforms which are activated by phosphatidylserine but not by Ca or DAG.

In a classical model for PKC activation, interaction of an extracellular signal with a receptor leads to formation of DAG and inositol triphosphate from cell membrane phosphatidylinositol, which with a concomitant increase in intracellular Ca^{2+} results in translocation of PKC from the cytosol to the membrane and subsequent activation of the enzyme (Nishizuka, 1984). The events in the signal transduction proceed very rapidly and induce PKC activity only temporarily. Long-term modulation of PKC activity requires sustained production of PKC activators in cells, such as DAG via phospholipase D-mediated breakdown of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) or free fatty acid and lyso-PC via phospholipase A-mediated breakdown of PC (Nishizuka, 1995). This sustained PKC activation has been proposed to play an important role in the aetiology of colon cancer (Blobe *et al*. 1994). In spite of the abundant *in vitro* data on PKC activation, little is known about the factors that regulate, and are able to modulate, PKC activity *in vivo*.

Dietary fat may affect colonic PKC activity by modifying phospholipid fatty acid composition of mucosal cells, which

Abbreviations: DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PKC, protein kinase C.

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in turn may modulate the endogenous activators of PKC. Alternatively, dietary fat may affect levels of PKC activators in faeces. Colonic contents contain measurable amounts of DAG, mainly as a result of the action of colonic bacteria on dietary lipids (Morotomi *et al*. 1990). The luminal DAG produced is capable of entering colonocytes (Morotomi *et al*. 1991) and thus possibly activating PKC. High-fat diets have been proposed to increase concentrations of luminal fat and bile salts, which together lead to enhanced DAG production in the colon. Bile salts may also activate PKC directly (Huang *et al*. 1992).

In a previous study, we found that a high-fat butter diet, when compared with a low-fat sunflowerseed-oil diet used as a control, significantly increased membrane-associated PKC activity in rat colonic mucosa (Pajari *et al*. 1997). The rats fed on the high-fat rapeseed and sunflowerseed-oil diets, however, had no increase in their PKC activities, which were close to that in the control group. In the present study, we compared butter with coconut oil to see whether the earlier observation is typical of saturated fats in general or specific for fats rich in certain types of saturated fatty acids, i.e. palmitic (16:0) and stearic (18:0) acids. With respect to these fatty acids, butter has a substantial overlap with beef fat which has been demonstrated to promote carcinogen-initiated tumour development in rat colon (Reddy, 1992). One explanation for this tumour-promoting effect might be that dietary long-chain saturated fatty acids are less well absorbed in the small intestine and continue on to the colon (Monsma *et al*. 1996), which results in an increased excretion of fat and bile salts into faeces. Coconut oil, on the other hand, contains mainly lauric (12:0) and myristic (14:0) acids and has not enhanced colonic tumour formation in experimental animals (Reddy & Maeura, 1984). Therefore, it was of interest to study whether these two saturated fats differ in their effects on colonic PKC activity. Two levels of the saturated fats were chosen to study the influence of fat quantity on PKC activity. The other purpose of the study was to elucidate the role of mucosal phospholipid fatty acid composition as well as faecal DAG levels in regulating colonic PKC activity *in vivo*.

Materials and methods

Animals and diets

The study protocol was approved by the Laboratory Animal Ethics Committee of the Faculty of Agriculture and Forestry, University of Helsinki. Male Wistar rats (*n* 65, 5 weeks of age) were obtained from the National Laboratory Animal Centre, University of Kuopio, Finland, and housed in plastic cages (three per cage) in a room with a controlled temperature (20–22°) and a 12 h light–dark cycle. After 1 week of acclimatization on a commercial rodent laboratory chow, the rats were randomly divided into five dietary treatment groups (*n* 13 per diet). The compositions of the experimental diets are shown in Tables 1 and 2. The low-fat control group was fed on a basal diet providing 10 % energy from sunflowerseed oil. The other groups were fed on diets containing either butter or coconut oil at 10 % and 43 % energy. The saturated-fat diets provided 3 % energy from sunflowerseed oil in order to prevent symptoms of essential fatty acid deficiency. Animals were fed on their respective diets for 4 weeks with free access to the diet and water. Body weights were recorded weekly. During the fourth week, each animal was kept in a metabolic cage for 1 d to collect faeces. Faecal samples were frozen in liquid N_2 and stored at −70° until analysed for DAG content.

Tissue preparation and protein kinase C activity

At the end of the feeding period, the rats were killed by CO_2 asphyxiation. Colons were removed and divided into proximal and distal segments equal in length. The segments were cut open longitudinally, flushed clean with ice-cold PBS and the mucosa was scraped off with a microscope slide. In addition, the caecum was removed, opened, flushed clean and the mucosa was scraped off, frozen and kept at −70° until phospholipid fatty acid analysis. All the following procedures were done at 4°. The colonic mucosa was homogenized in 5 ml buffer (20 mM-Tris-HCl (pH 7·4), 2 mM-EDTA, 10 mM-EGTA, pH 7·4, 0·25 M-sucrose, 1 mM-phenylmethylsulfonyl fluoride, 0·1 g leupeptin/l) by sonicating for

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

Diet …	Low-fat control	Butter		Coconut oil	
Ingredient		Low-fat	High-fat	Low-fat	High-fat
Dextrose	636	636	386	636	386
Casein	242	242	300	242	300
DL-Methionine	3	3	4	3	4
L-Arginine	2	2	3	2	3
Sunflowerseed oil	44	13	16	13	16
Butter	–	31	218	–	–
Coconut oil	–	–	–	31	218
Choline chloride	3	3	3	3	3
Minerals*	60	60	60	60	60
Vitamins†	10	10	10	10	10
Percentage of energy from fat	10	10	43	10	43

* The mineral composition was (g/kg diet): $CaCO_3$ 5, $CaHPO_4$ 14, K_2HPO_4 11·1, NaCl 8·3, $MgSO_4.7H_2O$ 20·3, $FeSO_4.7H_2O$ 0·5, $MnSO_4.H_2O$ 0·23, and (mg/kg diet) $CuSO_4.5H_2O$ 39·3, $ZnCl_2$ 37·6, KI 0·3.

† The vitamin composition was (mg/kg diet): calcium pantothenate 12, nicotinic acid 10, pyridoxine 6, riboflavin 5, thiamin 4, folic acid 0·5, biotin 0·2, DL-α-tocopherol acetate 66, all-*trans*-retinylacetate 2·5 (7000 IU), dextrose 894, and (μg/kg diet) menadione 60, cholecalciferol 7·5, cyanocobalamin 5.

Table 2. Fatty acid composition of the experimental diets (g/100 g total fatty acids)

Diet …	Low-fat control	Butter		Coconut oil	
Fatty acid		Low-fat	High-fat	Low-fat	High-fat
8:0	–	0·7	0·9	3·7	5·4
10:0	–	1·7	2·6	3·7	5·2
12:0	–	2·4	3·3	32·1	44·3
14:0	–	8·9	11·8	13·1	17·7
14:1*n*-5	–	0·8	1·1	–	–
16:0	6·2	26·0	33·4	8·9	9·5
16:1*n*-7	0·4	1·2	1·3	–	–
18:0	4·2	10·6	16·5	3·6	2·9
18:1*n*-9	23·9	25·4	21·1	12·8	8·2
18:2*n*-6	63·0	20·7	6·8	21·3	6·6
18:3*n*-6	–	0·3	0·4	–	–
18:3*n*-3	0·3	0·3	0·5	–	–
20:0	0·2	–	–	–	–
20:2*n*-6	0·4	–	–	–	–
22:0	0·7	0·2	–	0·2	–
22:1*n*-9	0·7	0·8	0·3	0·6	0·2
SFA	11·3	50·5	68·5	65·3	85·0
MUFA	25·0	28·2	23·8	13·4	8·4
PUFA	63·7	21·3	7·7	21·3	6·6

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

30 s on ice. The homogenate was centrifuged at 100 000 ***g*** (Beckman ultracentrifuge, Palo Alto, CA, USA) for 1 h and the supernatant fraction was collected and used as the PKC cytosolic fraction. The pellet was resuspended in 5 ml homogenization buffer containing 2 ml Triton X-100/l, incubated for 20 min and centrifuged at 100 000 ***g*** for 1 h. The resulting supernatant fraction contained the PKC membrane fraction. The crude cytosolic and membrane fractions were purified by DEAE-Sephacel chromatography (Pharmacia Biotech, Uppsala, Sweden) as follows: the DEAE-Sephacel gel was equilibrated before use in buffer A (20 mM-Tris-HCl (pH 7·5), 0·5 mM-EDTA, 0·5 mM-EGTA, 10 mM-2-mercaptoethanol); 2 ml cytosolic fraction or 3 ml membrane fraction was applied to a 0·75 ml DEAE-Sephacel gel and incubated for 1 h with gentle mixing. The gel was washed four times with 2 ml buffer A and PKC was then eluted with 2 ml buffer A containing 0·35 M-NaCl. The protein concentrations of the PKC fractions were measured using a Bio-Rad protein assay reagent (Bradford; Bio-Rad, Hercules, CA, USA) with bovine serum albumin (Sigma, St Louis, MO, USA) as a standard.

PKC activities of partially purified cytosolic and membrane fractions were measured using the vesicle assay described by El Touny *et al.* (1990) with modification. The reaction mixture of 250 µl contained 20 mM-Tris-HCl (pH 7·5), 0·4 mM-$CaCl_2$, 0·2 mg histone/ml (type III-S, Sigma), 40 µg phosphatidylserine/ml and 2 µg 1,2-dioleylglycerol/ml (Avanti Polar Lipids, Alabaster, AL, USA) in vesicles, 10 mM-MgCl, 10 µM-[γ-^{32}P]ATP (Amersham Pharmacia, Amersham, Bucks., UK), and 25 µl sample. The mixture was incubated at 34° for 10 min and the reaction was stopped by addition of 1 ml cold 2·31 M-TCA, followed by 1 ml bovine serum albumin (0·5 mg/ml) as a carrier. After a 5 min incubation, precipitates were collected onto glass fibre filters (Whatman GF/C, Maidstone, Kent, UK) by vacuum filtration and washed twice with 7 ml 0·93 M-TCA. Filters were air-dried for 10 min and moved into scintillation vials with 10 ml scintillation liquid (HiSafe3, Wallac, Turku, Finland). Radioactivities of the filters were counted by a liquid scintillation counter (Wallac). PKC activity is expressed as pmol ^{32}P incorporated/min per mg protein. The enzyme activity measured without the presence of the activators comprised 6–10 % of the total PKC activity. An addition of 50 µM-myristylated octapeptide (Bachem, Bubendorf, Switzerland) to the reaction mixture inhibited enzyme activity by 87 % and 94 % in cytosolic and membrane fractions respectively.

Fatty acid composition of phospholipids

Caecal total lipids were extracted (Folch *et al.* 1957) and phospholipids were fractionated by TLC (silica gel 60, Merck, Darmstadt, Germany) with a solvent system of chloroform–methanol–acetic acid–water (50 : 37·5 : 3·5 : 2, by vol.). Afterwards the TLC samples were methylated (Stoffel *et al.* 1959), and the fatty acid composition of phospholipid fractions was analysed using a Hewlett Packard 5890 Series II gas chromatograph (Little Falls, DE, USA) equipped with a fused silica capillary column (NB-351; Nordion, Helsinki, Finland) and a u.v. detector. The oven temperature was programmed to rise from 170° to 230° at a rate of 4°/min and then kept at 230° for 8 min. Fatty acid peaks were identified by comparison with a fatty acid methyl ester standard (GLC-91; Nu-Chec-Prep, Elysian, MN, USA). The proportions of individual fatty acids are expressed as percentages of the total fatty acids.

Faecal diacylglycerol

Faecal samples were extracted by a modified method of Bligh & Dyer (1959) as follows: 0·1 g sample was homogenized in 2 ml PBS. Then, 100 µl 1 M-NaCl and 750 µl chloroform–methanol (1 : 2, v/v) were added to 100 µl homogenate and vortexed thoroughly. An additional 250 µl 1 M-NaCl and 250 µl chloroform were added to the mixture and the phases

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were separated after centrifugation at 5000 **g** for 2 min. The chloroform phase was dried under N_2 and subsequently used for analysing the total amount of DAG by a commercial kit (Amersham Pharmacia) based on the *E. coli*-DAG-kinase method (Preiss *et al.* 1986). After the enzymic reaction the samples were purified by Amprep Si-columns (Amersham Pharmacia) according to manufacturer's instructions before scintillation counting.

Statistics

A one-way ANOVA was used to determine significant differences in body weights, PKC activity, fatty acid composition of phospholipids, and DAG concentration between the groups. When the P value was $<0{\cdot}05$, means were separated using Tukey's *post hoc* test. The difference in PKC activities between the proximal and distal colon was analysed using paired samples t test. The relationship between PKC activity and phospholipid fatty acids or faecal DAG was analysed by a linear regression analysis. The Systat statistical computer package (version 7.0, SPSS Inc., Chicago, IL, USA) was utilized for all the statistical analysis.

Results

No significant differences were found in body weights of the animals between the groups throughout the experiment. The mean values of the final body weights in different dietary groups were as follows (g): the control group 327 (SD 27), the low-fat butter group 326 (SD 26), the high-fat butter group 343 (SD 42), the low-fat coconut-oil group 313 (SD 39), and the high-fat coconut-oil group 338 (SD 25).

Dietary fat had no significant effect on cytosolic, membrane, or total PKC activity or membrane : cytosol ratio of the enzyme in either subside of the colon (Table 3). Although not significant, increasing amounts of both butter and coconut oil slightly increased the membrane PKC activity in the distal colon when compared with the control diet.

PKC activities differed between subsides of the colon (Table 4). The membrane and the total PKC activities were significantly higher in the distal than in the proximal colon ($P=0{\cdot}008$ and $P=0{\cdot}002$ respectively). The same phenomenon could also be seen with respect to the cytosolic PKC ($P=0{\cdot}054$). Membrane : cytosol ratio of the enzyme did not differ between subsides of the colon.

The fatty acid composition of caecal PC was relatively little affected by the experimental diets (Table 5). Ingestion of the high-fat coconut-oil diet resulted in a significant increase in 14:0 ($P=0{\cdot}0001$) when compared with the control or high-fat butter diet. The butter diet increased 18:1n-9 ($P=0{\cdot}003$) as well as total monounsaturated fatty acids ($P=0{\cdot}014$) when compared with the control diet.

There were more dietary-induced changes in fatty acid composition of caecal PE than in PC (Table 6). Common for the saturated fat diets was an increase in total saturated fatty

Table 3. Protein kinase C activity (pmol/min per mg protein) in colonic mucosa of rats fed on low-fat or high-fat butter or coconut-oil diets*

(Mean values with their standard errors for thirteen observations)

Diet …	Low-fat control		Low-fat butter		Low-fat coconut oil		High-fat butter		High-fat coconut oil	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Proximal colon										
Cytosol	604	63	600	50	795	132	629	56	609	41
Membrane	2080	100	2090	169	2140	190	2192	197	1935	139
Total (cytosol + membrane)	2684	120	2690	178	2935	267	2821	217	2544	137
Membrane : cytosol ratio	3·92	0·48	3·74	0·41	3·38	0·48	3·80	0·46	3·40	0·37
Distal colon										
Cytosol	766	77	616	68	803	115	701	55	678	65
Membrane	2083	150	2242	185	2290	153	2386	151	2312	201
Total (cytosol + membrane)	2850	179	2858	219	3093	243	3087	178	2990	225
Membrane : cytosol ratio	2·97	0·30	4·03	0·46	3·63	0·56	3·63	0·35	3·80	0·50

* For details of diets and procedures see Tables 1 and 2 and pp. 412–413.

Table 4. Comparison of protein kinase C activity (pmol/min per mg protein) between subsides of the colon†

(Mean values with their standard errors for sixty-five observations)

	Proximal colon		Distal colon		
	Mean	SE	Mean	SE	P value*
Cytosol	647	34	713	35	0·054
Membrane	2087	71	2263	74	0·008
Total (cytosol + membrane)	2735	85	2976	92	0·002
Membrane : cytosol ratio	3·65	0·19	3·61	0·20	0·874

* Data were analysed by paired samples t test.
† For details of procedures see pp. 412–413.

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Table 5. Fatty acid composition (g/100 g total fatty acids) of caecal mucosal phosphatidylcholine from rats fed on high-fat butter or coconut-oil diets*

(Mean values with their standard errors for six to eight observations)

Diet …	Low-fat control		High-fat butter		High-fat coconut oil	
Fatty acid	Mean	SE	Mean	SE	Mean	SE
14:0	1·77[a]	0·42	2·85[a]	0·27	6·91[b]	0·67
16:0	51·06	5·31	46·35	3·63	50·05	1·47
16:1*n*-7	2·44	0·25	2·11	0·49	2·16	0·21
18:0	17·85	1·80	14·97	1·10	15·00	0·82
18:1*n*-9	13·56[a]	1·14	23·13[b]	2·38	17·01[ab]	0·99
18:2*n*-6	6·12	1·49	6·88	1·60	6·56	1·56
20:3*n*-6	1·32	0·27	1·48	0·30	1·36	0·37
20:4*n*-6	1·60	0·65	1·00	0·31	0·93	0·36
SFA	70·69	4·66	64·17	4·84	72·00	2·64
MUFA	16·0[a]	1·33	25·24[b]	2·80	19·17[ab]	1·16
PUFA	9·03	1·87	9·35	1·77	8·84	1·88

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures see Tables 1 and 2 and pp. 412–413.

Table 6. Fatty acid composition (g/100 g total fatty acids) of caecal mucosal phosphatidylethanolamine from rats fed on high-fat butter or coconut-oil diets*

(Mean values with their standard errors for six to eight observations)

Diet …	Low-fat control		High-fat butter		High-fat coconut oil	
Fatty acid	Mean	SE	Mean	SE	Mean	SE
14:0	2·22[a]	0·31	6·39[a]	0·43	11·80[b]	2·85
16:0	27·63	0·70	29·44	0·85	24·26	2·40
16:1*n*-7	8·13[a]	0·79	4·54[b]	0·25	4·35[b]	0·43
18:0	7·48	1·51	10·42	0·97	11·75	2·62
18:1*n*-9	28·65[a]	1·11	34·16[b]	0·89	25·94[a]	0·73
18:2*n*-6	20·57[a]	1·23	10·15[b]	0·61	12·65[b]	1·47
20:3*n*-6	0·61	0·21	1·03	0·31	3·43	2·09
20:4*n*-6	4·70	1·30	3·83	0·78	5·80	1·55
SFA	37·34[a]	0·98	46·25[b]	0·54	47·81[b]	2·73
MUFA	36·80[a]	1·81	38·73[a]	1·06	30·28[b]	0·86
PUFA	25·88[a]	1·25	15·01[b]	1·49	21·88[a]	3·42

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures see Tables 1 and 2 and pp. 412–413.

acids ($P = 0·001$ for the coconut-oil group and $P = 0·006$ for the butter group), and a decrease in 16:1*n*-7 ($P = 0·0001$) as well as in 18:2*n*-6 ($P = 0·0001$) when compared with the control diet. Animals in the coconut-oil group had a higher proportion of 14:0 ($P = 0·001$) and a lower proportion of total monounsaturated fatty acids ($P = 0·005$) than those in the control group. The butter diet resulted in an increase in 18:1*n*-9 ($P = 0·001$) and a decrease in total polyunsaturated fatty acids ($P = 0·010$) when compared with the control diet.

PKC activity was associated with PC fatty acids so that saturated fatty acids 14:0 and 18:0 were positively correlated, while unsaturated 18:2*n*-6, 20:4*n*-6 and 16:1*n*-7 were negatively correlated, with membrane PKC activity in the distal colon (Fig. 1). These associations could also be seen in the proximal colon although they were not as significant as in the distal colon (results not shown). Nearly similar correlations were found with respect to total PKC activity, whereas cytosolic PKC was not associated with PC fatty acids. No significant correlations were found between PE fatty acids and PKC activity.

Table 7. Faecal diacylglycerol concentration (ng/g wet weight) of rats fed on high-fat butter or coconut-oil diets*

(Mean values with their standard errors for thirteen observations)

	Faecal diacylglycerol concentration	
Diet	Mean	SE
Low-fat control	181	13
High-fat butter	154	10
High-fat coconut oil	173	12

* For details of diets and procedures see Tables 1 and 2 and pp. 412–413.

Faecal DAG concentration was not significantly ($P = 0·254$) affected by the type or quantity of dietary fat (Table 7). There was no correlation between PKC activity and faecal DAG.

Discussion

In an earlier study, we found that a high-fat butter diet significantly increased membrane PKC activity in rat colon

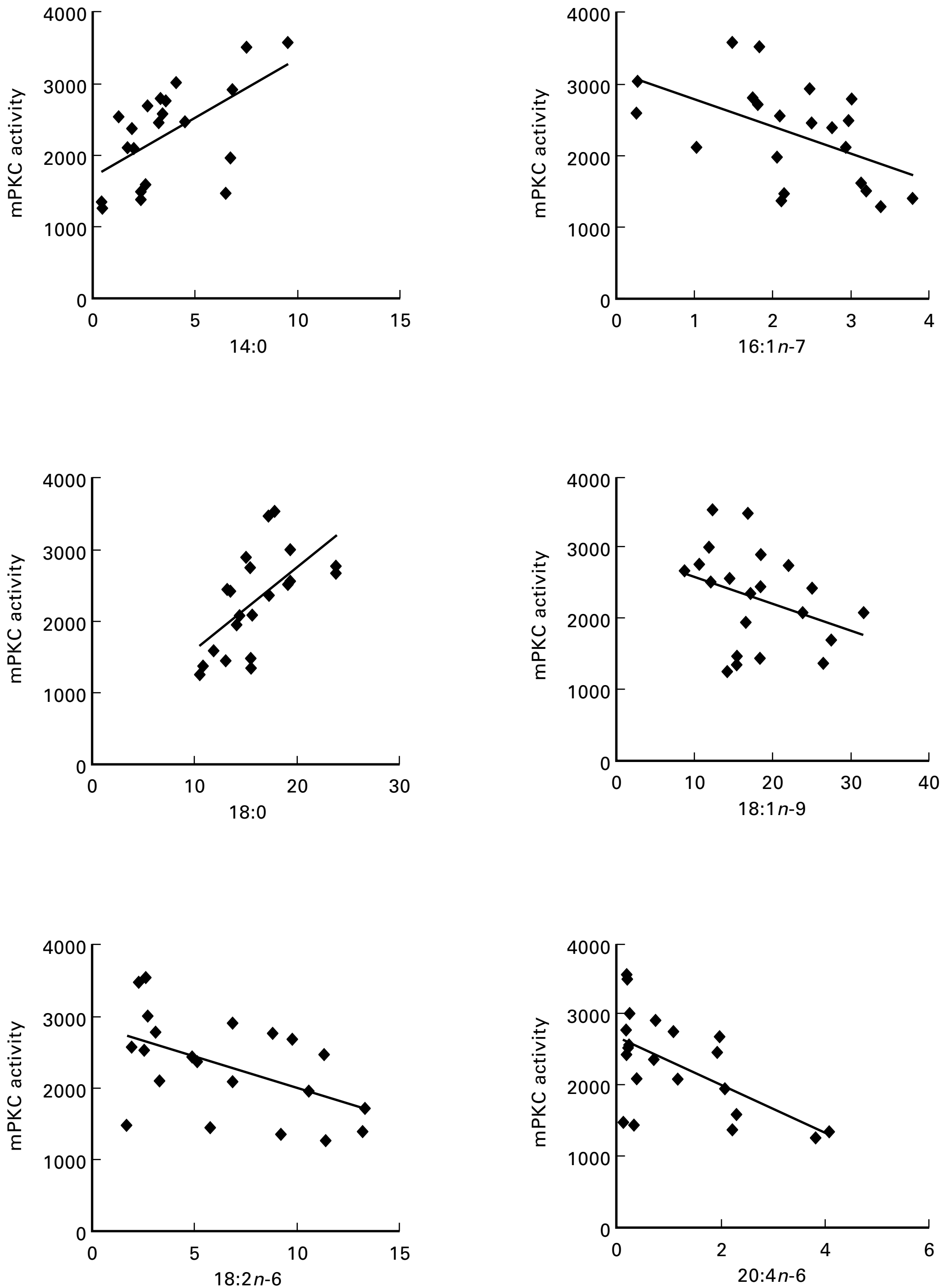

Fig. 1. Relationships between proportions of fatty acids of phosphatidylcholine (g/100 g total fatty acids) and membrane protein kinase C (mPKC; pmol/min per mg protein) in the distal colon of rats fed on the experimental diets. For details of diets and procedures see Tables 1 and 2 and pp. 412–413. mPKC activity *v.* 14:0, *r* 0·58 and *P*=0·006; *v.* 16:1*n*-7, *r* 0·52 and *P* = 0·016; *v.* 18:0, *r* 0·54 and *P* = 0·003; *v.* 18:1*n*-9, *r* 0·34 and *P*=0·123; *v.* 18:2*n*-6, *r* 0·52 and *P*=0·01; *v.* 20:4*n*-6, *r* 0·59 and *P*=0·005.

when compared with a low-fat sunflowerseed-oil diet used as a control (Pajari *et al.* 1997). These data were consistent with two other *in vivo* studies in which saturated beef-tallow diets also resulted in an increase in colonic membrane PKC activity (Chapkin *et al.* 1993; Lafave *et al.* 1994). The entire colonic mucosa was used for PKC analysis in our earlier study. Because effect of fat may be site specific, PKC activity in the present study was analysed from the proximal and distal colons separately. We expected to see a pronounced effect of the high-fat butter diet on PKC activity in the distal

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colon, since butter and beef tallow have been shown to exert their promotive effect on cell proliferation mainly in the distal colon (Lee *et al*. 1993). Cell proliferation, in turn, has been correlated with colonic PKC activity (Chapkin *et al*. 1993). We were, however, unable to see such an effect in the present study. What could be seen was that both butter and coconut-oil diets slightly increased the membrane PKC activity in the distal colon as the level of fat in the diets increased. With respect to the high-fat butter diet, the effect seen was much weaker than in our earlier study (Pajari *et al*. 1997). Because the compositions of the high-fat butter and low-fat control diets were the same in both studies, there is no clear explanation for the discrepancy in the data.

Levels of PKC activity, both cytosolic and membrane associated, differed between the subsides of the colon so that PKC activity was significantly higher in the distal than in the proximal colon. This may be due to differences in expression of PKC isozymes between the subsides of the colon (Davidson *et al*. 1995) or due to longer exposure time to luminal contents in the distal colon.

A number of studies have shown that the fatty acid composition of colonic mucosal phospholipids, at least partially, reflects the composition of dietary fat (Robblee *et al*. 1988; Lee *et al*. 1993; Rao *et al*. 1993). In the present study, the composition of two major phospholipid classes, PC and PE, was analysed from caecal mucosa in order to see whether diet could regulate colonic PKC activity through modulating phospholipid fatty acid composition. In general, dietary effects were more obvious in the PE fraction, whereas the PC fatty acid composition appeared to be more resistant to dietary induced changes. Because PC particularly has been associated with regulation of PKC activity (Nishizuka, 1995), the resistance of PC to diet could partly explain the relatively small effects of the dietary fats on PKC activity in this study.

Interestingly, PC fatty acids were significantly correlated with membrane PKC activity in both subsides of the colon. It has been proposed that PC in particular is involved in regulating sustained PKC activation in cells, which is required for long-term cellular responses such as cell proliferation and differentiation (Nishizuka, 1995). Our data concord with this view in suggesting that the ability of PC to support PKC activity is related to the fatty acid composition of PC. Of PC fatty acids, saturated fatty acids (14:0 and 18:0) were associated with increased, and unsaturated fatty acids (16:1*n*-7, 18:2*n*-6, and 20:4*n*-6) were associated with decreased, membrane PKC activity. These associations could be mediated by several alternative mechanisms. One such mechanism could be hydrolysis products of PC such as DAG, free fatty acids, and lyso-PC which are known enhancers of PKC activity *in vitro* (Nishizuka, 1995). The direction of the associations is, however, difficult to explain because saturated DAG and saturated fatty acids have generally not shown a stimulatory effect on PKC activity *in vitro* (Hannun *et al*. 1986). Unsaturated fatty acids have, on the other hand, resulted in a biphasic effect on PKC activity (Holian & Nelson, 1992; Lo *et al*. 1994), with higher concentrations depressing it. The *in vitro* data may not necessarily be applicable to the *in vivo* situation because the observations made in *in vitro* systems could have been partly dependent on the techniques and activators used for PKC activity measurement.

An alternative explanation for the association between PC fatty acids and PKC activity may be related to effects of fatty acids on membrane physical properties. This is supported by a study in which increasing PC unsaturation in PC vesicles resulted in an increase in PKC activity, whereas including PE in the vesicles resulted in an opposite effect, i.e. increasing PC unsaturation in the presence of PE decreased PKC activity (Slater *et al*. 1994). The authors attributed these effects to the biophysical state of the membrane bilayer structure which is determined by a complex interaction of head groups and unsaturation level of membrane phospholipids. With respect to the physical variables measured, PKC appeared to have a narrow optimal value for its maximum activity (Slater *et al*. 1994). The precise mechanism by which PC fatty acids affect PKC activity remains to be determined.

Faecal DAG produced by the action of colonic bacteria on dietary lipids is proposed to be one of the key factors which regulates colonic PKC activity (Morotomi *et al*. 1990, 1991). In the present study, no difference in faecal DAG concentration was found between the low-fat and high-fat dietary groups or between the groups fed on different types of saturated fat. This result does not support the theory that high intake of fat or high intake of long-chain saturated fatty acids (16:0 and 18:0) would lead to increased levels of faecal lipids and thus enhanced production of DAG in the colon. In earlier studies, a very high intake of butter (i.e. 260 g/kg diet) has been required to significantly increase faecal lipids (Kim *et al*. 1998), whereas lower intakes (140 or 200 g/kg diet) have been without effect (Awad *et al*. 1989; Chang *et al*. 1994 respectively).

The finding that PKC activity was not correlated with faecal DAG in either subside of the colon was unexpected. This might indicate that faecal DAG was not taken up by colonocytes to a significant extent or that if taken up it was metabolized through pathways not related to PKC activation (Florin-Christensen *et al*. 1992). Dietary fat is known to affect fatty acid composition of faecal DAG (Pickering *et al*. 1995). Faecal DAG composition was not measured in the present study, so it is difficult to say whether the lack of association was due to some specific effects of the experimental diets on DAG composition.

In summary, the results of the present study suggest that high-fat saturated diets do not increase faecal DAG concentration or PKC activity in any region of the colon. Ingestion of saturated fats resulted in moderate changes in the fatty acid composition of caecal phospholipids, which were more obvious in the PE than in the PC fraction. The fatty acid composition of PC was significantly correlated with membrane PKC activity, supporting the involvement of PC in regulating PKC activity *in vivo*.

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