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A high-beef diet alters protein kinase C isozyme expression in rat colonic mucosa

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*We recently reported that a red meat (beef) diet relative to a casein-based diet increases protein kinase C (PKC) activity in rat colonic mucosa. The purpose of this study was to further elucidate the effects of a high-beef diet on colonic intracellular signal transduction by analyzing steady-state protein levels of different PKC isozymes as well as activities of the three types of sphingomyelinases. Male Wistar rats (*n = *12/group) were fed AIN93G-based diets either high in beef or casein for 4 weeks. Rats fed the beef diet had significantly (*P < *0.05) higher cytosolic PKC* α *and lower membrane PKC* δ *protein levels than rats fed the casein diet. The beef-fed rats also had alterations in subfractions of PKC* ζ/λ *so that they had a significantly (*P = *0.001) lower level of membrane 70 & 75 kDa fraction and a higher (*P = *0.001) level of cytosolic 40 & 43 kDa fraction than rats fed the casein diet. Because protein levels analyzed with a PKC* ζ*-specific antibody were similar, these differences in PKC* ζ/λ *were probably due to changes in PKC* λ *expression. PKC* β*2 levels did not differ between the dietary groups. Diet had no significant effect on the activity of acid, neutral, or alkaline sphingomyelinase. This study demonstrated that consumption of a high-beef diet is capable of modulating PKC isozyme levels in rat colon, which might be one of the mechanisms whereby red meat affects colon carcinogenesis.* (J. Nutr. Biochem. 11: 474–481, 2000) *© Elsevier Science Inc. 2000. All rights reserved.*

Keywords: red meat; protein kinase C; sphingomyelinase; rat colon

Introduction

Most case-control studies and some, though not all, cohort studies have shown an elevated colon cancer risk for those consuming diets high in red or processed meat (reviewed in Refs. 1 and 2). Several mechanisms mediating the promoting effect of meat on colon carcinogenesis have been proposed but experimental studies confirming these mechanisms are sparse. We recently reported that feeding a high-beef diet compared with a casein diet significantly increases PKC activity in rat colonic mucosa.[3] PKC plays an important role in cellular signal transduction machinery which, along with the mutations in oncogenes and tumor suppressor genes, may contribute to the neoplastic process leading to tumor formation. Colonic tumors from both human and animal origin have reduced PKC activity as well as alterations in levels of several PKC isoenzymes as compared with the surrounding uninvolved mucosa.[4–9] Changes in PKC activity and isozyme expression have been demonstrated to occur prior to tumor formation and involve a persistent activation/translocation and subsequent down-regulation of the enzyme.[6,10,11] This indicates involvement of PKC isozymes at early stages of the neoplastic process.

At least 11 different PKC isozymes have been identified, of which colonic epithelial cells express α, β/β2, δ, ϵ, ζ, and ι/λ.[12–15] These PKC isozymes differ with respect to activator requirements so that PKC α and β/β2 are conventional isoforms regulated by Ca^{2+}, diacylglycerol (DAG), and phosphatidylserine (PS). PKC δ and ϵ are novel isoforms activated by DAG and PS, whereas PKC ζ and ι/λ are

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atypical isoforms stimulated by PS but not by Ca^{2+} or DAG. This variation in activators as well as subcellular localization enable PKC isozymes to mediate a wide range of signals regulating cellular functions such as cell proliferation and differentiation,[16] cell cycle control,[17,18] intracellular trafficking,[19] or even apoptosis.[20]

Sphingomyelinases (SMases) are members of a recently discovered signal transduction pathway related to cell growth suppression (reviewed in Ref. 21). Activation of SMases results in hydrolysis of sphingomyelin and formation of ceramide, a putative lipid second messenger. Ceramide has been shown to induce apoptosis and cell cycle arrest[22,23] as well as inhibit cell proliferation and stimulate cell differentiation.[24,25] Three types of SMases differing with respect to pH optimum and cellular localization have been identified: Acid SMase is a lysosomal enzyme,[26] neutral SMase is a plasma membrane-associated enzyme,[27] and alkaline SMase is a brush border-associated enzyme.[28] The activities of all the three types of SMases have been reduced in colorectal carcinomas,[29] indicating that SMases may have an active role in colonic malignant transformation. A number of studies suggest that interaction between the sphingomyelin pathway and PKC isozyme occurs. Specifically, ceramide has been reported to inactivate PKC α and possibly other conventional isoforms as well, probably by causing dephosphorylation of the enzyme.[30] On the other hand, ceramide produced by acid SMase may have a specific role in activating atypical PKC ζ.[31,32]

In the present study, we extended our earlier finding by studying the effects of a high-beef diet on PKC isozyme expression and intracellular localization in rat colon. The PKC isozymes analyzed were PKC α, β2, δ, and ζ/λ, which are the isoforms most consistently found in human and rat colons.[12–14] To elucidate the possible interactions between PKC and the sphingomyelin pathway, activities of the three types of sphingomyelinases were also analyzed.

Methods and materials

Animals and diets

The protocol for this experiment was approved by the Laboratory Animal Ethics Committee of the Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland. Twenty-four male Wistar rats were obtained from the University Experimental Animal Facility (Helsinki, Finland). They were maintained in plastic cages (3 rats per cage) in a room with a controlled temperature (20–22°C) and a 12-hr light/dark cycle for the duration of the study. After one week of acclimatization on a standard rodent chow, the animals were divided into two dietary treatment groups equal in weight (12 rats/diet). They were fed the experimental diets for 5 weeks during which they had free access to the diets and tap water. Their body weights were recorded weekly.

The experimental diets were modified AIN-93G diets[33] with casein or beef as a protein source (*Table 1*). Before addition to the diet, the low-fat beef (*M. longissimus dorsi*) was minced and freeze-dried but not cooked because we wanted to exclude the possible effects of compounds such as heterocyclic amines formed during cooking. The composition of the freeze-dried beef was analyzed and it contained 21% fat and 73% protein. To obtain similar fat content and fatty acid composition of the diets, 50 g/kg beef tallow was added to the casein diet. The contents of iron, zinc, calcium, and potassium in the casein diet were adjusted to the same level as in the beef diet (*Table 1*). Based on the fatty acid analyses, the beef diet contained approximately 360 mg arachidonic acid per kg diet, which was not present in the casein diet. However, results of our previous study indicate that arachidonic acid at the level found in a beef diet does not affect either PKC or SMase activities in rat colon.[3]

Table 1 Composition of experimental diets expressed as g/1,000g of diet

Ingredients*	Diets	
	Casein	Beef
Casein	200	
Beef†		240
Dextrose	621	639
Vegetable oil mix‡	70	70
Beef tallow	50	
L-Cystine	3	3
Choline chloride	3	3
Mineral mix (AIN-93MX)	35	35
Additional mineral mix§	8	
Vitamin mix (AIN-93VX)	10	10
Tertiary butylhydroxyquinone	0.014	0.014

*Casein was obtained from Kainuun Osuusmeijeri (Sotkamo, Finland), beef and beef tallow from Pauli Vainio (Helsinki, Finland), dextrose from Six Oy (Helsinki, Finland), mineral and vitamin mix (AIN93) from Harlan Teklad (Madison, WI USA), L-cystine, choline chloride, and tertiary butylhydroxyquinone from Yliopiston Apteekki (Helsinki, Finland). Rapeseed and sunflower seed oils were from a local market

†Freeze-dried beef contained 21% fat and 73% protein.

‡Vegetable oil mix was composed of 45% rapeseed oil and 55% sunflower seed oil.

§Additional mineral mix supplied (g/kg diet) 7.65 K_2HPO_4, 0.19 $CaHPO_4$, 0.077 $FeSO_4$, and 0.083 $ZnCl_2$.

Tissue preparation and protein extraction

At the end of the feeding period, the rats were killed by CO_2 asphyxiation. Their colons were removed and cut open longitudinally. The colonic contents were collected into test tubes and the residues were rinsed out with ice-cold saline. The colons were divided into proximal and distal segments of equal length and the mucosa was scraped off with a microscope slide. The proximal mucosa and the colonic contents were snap frozen in liquid nitrogen and stored at −80°C until analyzed for SMase activity. The distal mucosa was used for PKC isozyme analysis. Cytosolic and membrane proteins from the mucosal sample were extracted as described previously.[3] Briefly, homogenized mucosa was ultracentrifuged at 100,000 × *g* for 1 hr, and the supernatant 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 hr. The resulting supernatant contained the membrane fraction. Protein concentration of the crude fractions was measured using a Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA USA) (Bradford) with bovine serum albumin (BSA) 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 retentate was mixed with an equal volume of sodiumdodecylsulphate (SDS)-sample buffer, boiled for 5 min and stored at −80°C until use. Rat brain homogenate containing both cytosolic and

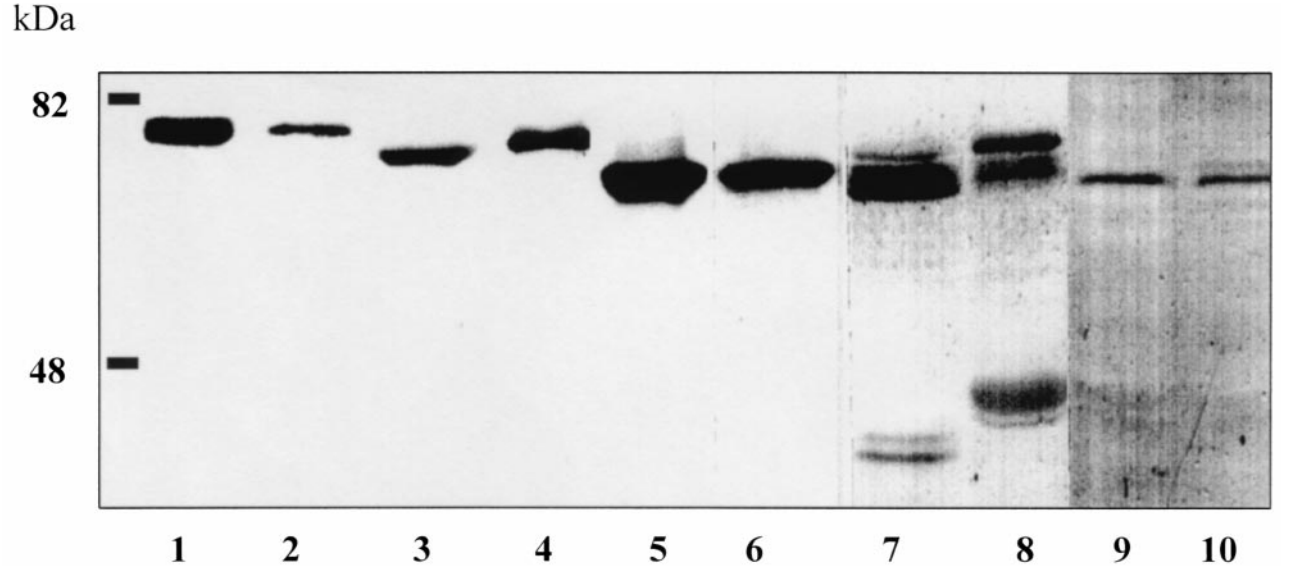

Figure 1 Representative Western blots of protein kinase C (PKC) isozymes in rat colonic mucosal and brain samples. Lanes are as follows: PKC α in colon (1) and in brain (2), PKC β2 in colon (3) and in brain (4), PKC δ in colon (5) and in brain (6), PKC ζ/λ in colon (7) and in brain (8), PKC ζ in colon (9) and brain (10). Western blots were conducted as described in the Methods and materials section.

membrane proteins was used as a control for PKC antibody specificity in immunoblotting analysis. Rat brain homogenate was prepared essentially in the same way as described for the colon samples. Colonic mucosa samples (30–100 μg) and rat brain homogenate (1–5 μg) were subjected to 10% SDS-PAGE and then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA USA) at 210 mA for 3 hr. Transfer of proteins to PVDF membranes was confirmed by staining gels with 0.1% Coomassie Brilliant Blue R-250 in fixative (40% methanol, 10% acetic acid). The membranes were blocked with 1% nonfat dry milk in PBS at 4°C overnight, washed with 0.5% BSA (Sigma, St. Louis, MO) in PBS, and incubated with antibodies for PKC isozymes in 1% BSA in PBS. PKC bands were visualized by colorimetric staining of blots with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium substrate mix (Bio-Rad Laboratories, Hercules, CA USA). Blots were scanned on a Sharp JX325 Scanner and the scanning images were analyzed with ImageMaster®1D Software, version 2.0 (Pharmacia Biotech, Uppsala, Sweden). The results of duplicate samples 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 gels to ensure that the colorimetric signal was quantitatively detectable.

We analyzed protein levels of PKC α, β2, δ, ζ/λ, and ζ in rat colonic mucosa. Dilution of individual primary antibody and alkaline phosphatase-conjugated secondary antibody was optimized for each PKC isozyme. The antibody for the PKC α reacts, to a lesser extent, with β isozyme. PKC β2 and δ antibodies are non-cross-reactive with other PKC isoforms. Blocking peptide of PKC ζ/λ was used to control the specificity of bands given by the PKC ζ/λ antiserum. For this blocking test, a 10-fold (by weight) excess of peptide antigen was incubated with PKC ζ/λ antibody in 1% 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 α and δ were purchased from Transduction Laboratories (Lexington, KY USA) and alkaline phosphatase-conjugated anti-mouse secondary antibody was obtained from Bio-Rad Laboratories (Hercules, CA USA). Polyclonal rabbit anti-PKC β2, ζ/λ, ζ, blocking peptide for PKC ζ/λ, and alkaline phosphatase-conjugated anti-rabbit secondary antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA USA).

Sphingomyelinase activity

The activities of the three types of SMases from the mucosal samples were analyzed essentially in the same way as described previously.[3] For alkaline SMase analysis, colonic contents were vacuum-dried and the weights of the dried feces were determined. The samples were then suspended in 0.15 M NaCl, followed by centrifugation at 3000 rpm for 10 min. The alkaline SMase activity in the supernatant was determined as described for the mucosal samples,[3] and the results were normalized per the fecal weights.

Statistical analysis

The data were analyzed by unpaired-samples *t*-test, with values of $P < 0.05$ considered to be significant. The correlation data were analyzed by a linear regression analysis. The SYSTAT statistical package (version 7.0, SPSS Inc., Chigaco, IL USA) was utilized for the statistical analysis.

Results

There were no differences between the dietary groups with respect to weight gain, final body weight, colon mucosal weight, or colon length. Cecal mucosal weight was greater in rats fed the beef diet than in rats fed the casein diet (0.21 ± 0.16 g vs. 0.16 ± 0.09 g, $P = 0.047$).

PKC isoforms α, β2, δ, ζ/λ, and ζ were analyzed from mucosal samples of rat distal colon. Representative immunoblots of each PKC isozyme are shown in *Figure 1*. Rat colon and rat brain (positive control) samples gave a single band for PKC α and β2 at approximately 80 and 74 kDa, respectively. PKC δ was detected as a 73 kDa band in brain and as a 72 & 69 kDa doublet in colon samples. Two different antibodies were used to study PKC ζ expression. The first antibody cross-reacts, to some extent, with PKC λ,

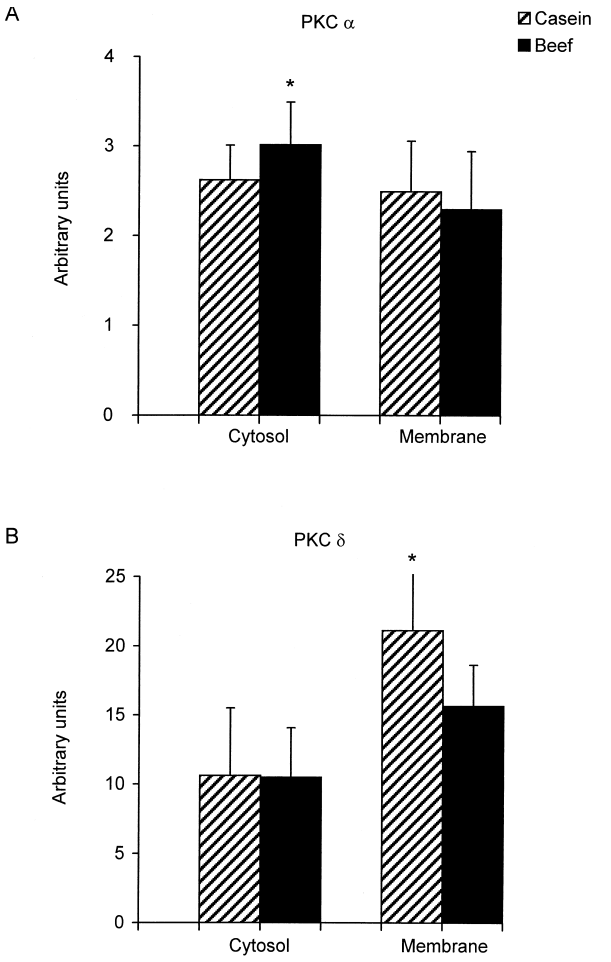

Figure 2 Protein kinase C α (PKC α) protein expression (Panel A) and PKC δ protein expression (Panel B) in colonic mucosa of rats fed a casein or beef diet. Values are means ± SD of 12 rats/group. Results are expressed as arbitrary units; that is, intensity (optical density × mm^2) of sample band divided by intensity of a rat brain control band. *$P < 0.05$.

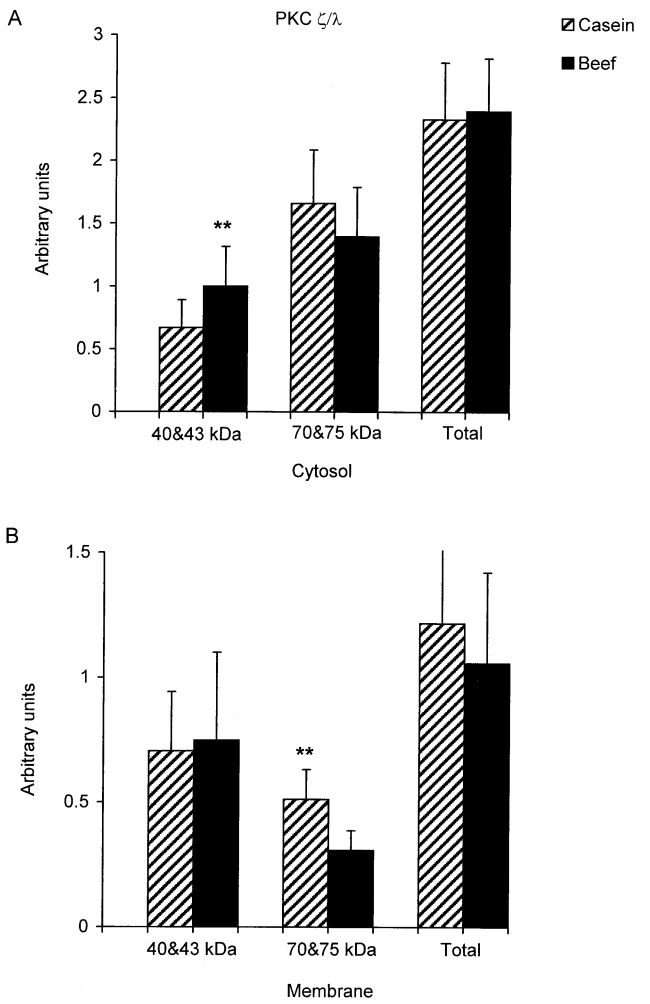

Figure 3 Protein kinase C ζ/λ (PKC ζ/λ) protein expression in colonic mucosa of rats fed a casein or beef diet. Values are means ± SD of 12 rats/group. Results are expressed as arbitrary units; that is, intensity (optical density × mm^2) of sample band divided by intensity of a rat brain control band. **$P < 0.01$.

which possesses a very close amino acid sequence homology with PKC ζ. This PKC ζ/λ antibody recognized three bands at 75, 70, and 50 kDa in brain, and doublet bands of 70 & 75 kDa and 40 & 43 kDa in distal colon samples, all of which were eliminated when the blocking peptide of PKC ζ/λ was preincubated with the antibody. Because rat colon has been found to express PKC λ,[15] we also utilized a recently available antibody specific for PKC ζ. This antibody has been directed against the aminoterminus of PKC ζ and therefore, it does not recognize the lower molecular weight bands typical of PKC ζ.[12,34]

Rats fed the beef diet had a higher ($P = 0.037$) level of cytosolic PKC α and a lower ($P = 0.025$) level of membrane PKC δ than rats fed the casein diet (*Figure 2*). Although the differences did not reach statistical significance, the membrane/cytosol ratios of both PKC α and δ were lower in rats consuming the beef diet than in those consuming casein (0.78 ± 0.28 vs. 0.95 ± 0.18, $P = 0.08$ for PKC α and 1.68 ± 0.61 vs. 2.28 ± 0.9, $P = 0.07$ for PKC δ). PKC ζ/λ expression was affected so that the beef-fed rats had a significantly ($P = 0.008$) higher level of cytosolic 40 & 43 kDa fraction and a lower ($P = 0.001$) level of membrane 70 & 75 kDa fraction than the casein-fed rats (*Figure 3*). In contrast, when using the PKC ζ-specific antibody, no difference in PKC ζ levels between the dietary groups could be seen (*Table 2*). There were no significant differences between the beef- and casein-fed rats in cytosolic PKC β2 levels (0.57 ± 0.24 vs. 0.48 ± 0.24, $P = 0.39$)

Table 2 Protein kinase C ζ (PKC ζ) expression in colonic mucosa of rats fed experimental diets*

PKC ζ†	Casein	Beef	*P*-Value
Cytosol	1.36 ± 0.46	1.40 ± 0.52	0.862
Membrane	2.24 ± 0.65	2.25 ± 0.45	0.971

*Values are means ± SD for 12 rats/group. Results are expressed as arbitrary units; that is, intensity (optical density × mm^2) of sample band divided by intensity of a rat brain control band.
†PKC ζ was analyzed by using a PKC ζ-specific antibody.

Table 3 Sphingomyelinase activities in colonic mucosa of rats fed experimental diets*

Sphingomyelinase	Casein	Beef	*P*-Value
Acid	190 ± 227	121 ± 44	0.314
Neutral	475 ± 192	524 ± 96	0.436
Alkaline	585 ± 60	584 ± 98	0.964
Alkaline in feces	147 ± 169	100 ± 121	0.484

*Values are means ± SD in pmol/(h · mg protein) for 12 rats/group.

or membrane PKC β2 levels (0.15 ± 0.07 vs. 0.16 ± 0.09, $P = 0.68$).

Dietary treatment had no significant effect on activities of mucosal acid, neutral, and alkaline SMases in the proximal colon (*Table 3*). Acid SMase activity was significantly correlated with the level of membrane PKC ζ analyzed by using the PKC ζ-specific antibody ($r = 0.65$, $P = 0.001$; *Figure 4A*) and inversely correlated with the level of total cytosolic PKC ζ/λ ($r = -0.49$, $P = 0.019$; *Figure 4B*). Neutral SMase activity showed a significant correlation with the 40 & 43 kDa fraction of cytosolic PKC ζ/λ ($r = 0.55$, $P = 0.006$; *Figure 4C*).

Discussion

This study demonstrates that a high-beef diet modulates protein levels and intracellular localization of colonic PKC α, δ, and ζ/λ while not affecting PKC β2 levels nor activities of acid, neutral, and alkaline SMases. These results extend our earlier study, which showed that a high-beef diet increases mucosal PKC activity in rat colon.[3] Alterations in both PKC activity and isozyme expression have been demonstrated to occur in different stages of colonic tumorigenesis in humans as well as in animals,[4–15] suggesting that PKC-mediated cell signal transduction pathways may be an important mechanism in colon carcinogenesis.

Rats fed the beef diet had a significantly lower level of membrane PKC δ and a higher level of cytosolic PKC α as well as a lower ($P < 0.08$) membrane-to-cytosol ratio of both the isozymes relative to rats fed the casein diet. Because association of PKC with membrane/cytoskeletal fraction is generally thought to be indicative of enzymatic activation, these results might suggest a decreased activation of PKC δ and perhaps PKC α in the beef-fed rats. Protein levels of both PKC α and δ are down-regulated in colonic tumors.[13,14] The two isozymes have also been associated with cell differentiation and growth arrest in a number of cell types[35,36] and the proteolytically activated PKC δ has been directly linked to the onset of apoptosis.[20,37] In an in vitro model of colon carcinogenesis, overexpression of PKC δ suppressed growth and reversed the transformed phenotype of the cells.[38] Studies with colon cancer cell lines support the role of PKC α in mediating colonic cell differentiation and/or growth arrest.[39–41] The possible mechanisms whereby PKC α affects these cellular processes may involve induction of expression of intercellular adhesion molecules[40] and/or a cell cycle regulatory protein, $p21^{waf1}$.[41,42] In view of these findings, the changes seen in PKC α and δ expression in our study may indicate decreased colonic differentiation in the rats fed the beef diet relative to rats fed the casein diet.

Interestingly, the dietary groups did not differ with respect to PKC β2 expression. PKC β2 has been linked to colonic proliferation[43,44] and its protein levels have been increased in colonic tumors as compared with surrounding mucosa,[7,11,14] suggesting a role for PKC β2 in malignant transformation. That there were no changes in PKC β2 levels may implicate that the beef diet had no direct effect on proliferation, at least not through PKC β2.

Atypical PKCs ζ and λ can be activated by different growth factors[45,46] and cytokines,[32,47] and they have been involved in mediating growth, differentiation, and maturation in several cell types.[34,46,48] In normal rat colonic epithelium, PKC ζ was found to be expressed predominantly in postmitotic cells of the upper crypt and surface mucosa, supporting the role for PKC ζ in mediating colonocyte differentiation.[49] In the present study, two different antibodies were utilized to study PKC ζ expression: The first antibody recognizes multiple bands and cross-reacts with PKC λ; the second one is specific for PKC ζ but recognizes only the high-molecular bands of PKC ζ (between 70 and 80 kDa). When the PKC ζ/λ antibody was used, rats fed the beef diet had a significantly lower level of the 70 & 75 kDa fraction and a higher level of the 40 & 43 kDa fraction than rats fed the casein diet. In contrast, when using the PKC ζ-specific antibody, protein levels of the high-molecular fraction were similar in the two dietary groups, suggesting that the differences seen with the PKC ζ/λ antibody may be due to PKC λ. The low-molecular bands are considered to be proteolytical products of PKC that lack the regulatory domain and are thus permanently active.[34] Therefore, it is possible that changes in levels of PKC ζ/λ subfractions could have accounted for the increased colonic PKC activity in rats fed the beef diet in our earlier study.[3] It is noteworthy that in some studies, increased proteolysis as well as persistent activation of PKC have been related to down-regulation of the enzyme.[6,10,50] Down-regulation of PKC ζ and λ is a feature of carcinogen-induced colonic tumors in rats,[11,14,15,51] which has been blocked by feeding fish oil,[11,15] a 1,25-dihydroxyvitamin D_3 analogue,[52] or the nonsteroidal antiinflammatory drug piroxicam.[51] In line with epidemiological evidence, each of these agents was also able to reduce colonic tumor formation. Thus, it appears that atypical PKCs are an important cellular pathway through which diet as well as specific pharmacological agents, at least partly, mediate their effects on colon carcinogenesis.

By which mechanism a high-beef diet affects colonic PKC isozyme expression was not addressed in the present study. The most plausible explanation would be enhanced luminal DAG production in rats fed the beef diet, but in our earlier study we could see no difference between the beef- and casein-fed rats in fecal DAG levels.[3] Bile acids have been reported to affect PKC isozymes in vitro,[53] but because the amount and quality of fat in the two diets were similar, one would not expect great differences in bile acid excretion between the rats maintained on the two diets. This issue is clearly in need of further studies.

Several lines of evidence suggest that sphingolipid sig-

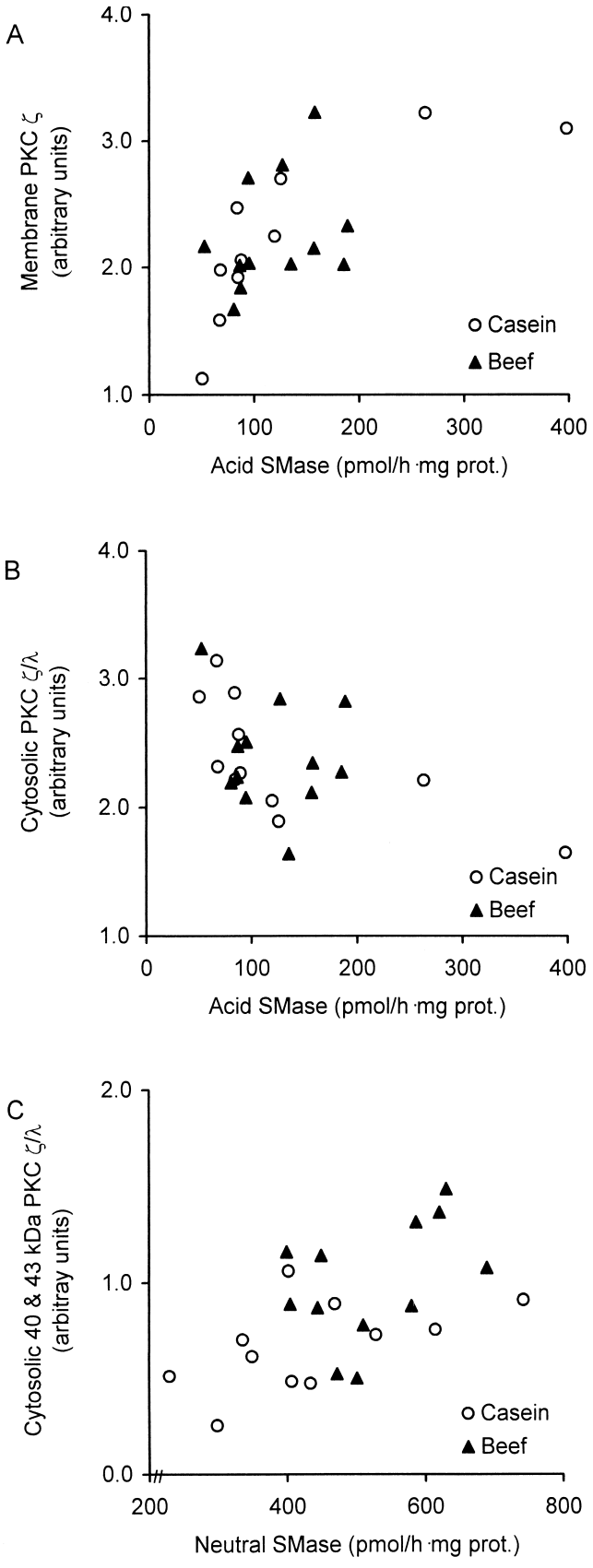

Figure 4 Relations between sphingomyelinase (SMase) activities and protein kinase C (PKC) ζ or ζ/λ protein levels in colonic mucosa of rats fed a casein or beef diet. Panel A: acid SMase activity and membrane PKC ζ analyzed with a PKC ζ-specific antibody ($r = 0.65$, $P = 0.001$); panel B: acid SMase activity and total cytosolic PKC ζ/λ ($r = -0.49$, $P = 0.019$); panel C: neutral SMase and the 40 & 43 kDa fraction of cytosolic PKC ζ/λ ($r = 0.55$, $P = 0.006$).

naling is involved in the colonic neoplastic process. First, SMases hydrolyze sphingomyelin to generate ceramide, which is a putative second messenger implicated in cell differentiation and induction of apoptosis.[21] Second, the activities of all three types of SMases have been decreased in human colorectal carcinomas as compared with the surrounding tissue.[29] Third, in mice treated with dimethylhydrazine, supplementation of diet with sphingomyelin has reduced the number of aberrant crypt foci and the appearance of adenocarcinoma in the colon.[54,55] As a source of sphingomyelin as well as other components of cell membranes,[56] beef might affect colonic sphingolipid signaling. In the present study, no difference in the activities of acid, neutral, and alkaline SMases between the dietary groups could be found. This may indicate that the beef diet was unable to modulate sphingomyelin pathway in the colon. However, it should be pointed out that the activities of SMases were determined from the proximal colonic mucosa. Whether the activity was changed in the distal part of the colon where tumorigenesis often occurs remains elusive. Because alkaline SMase may have a specific role in hydrolyzing diet-derived sphingomyelin in the colonic lumen,[21] its activity was analyzed from the colonic contents. Again, no dietary effect could be observed. Interestingly, the acid SMase activity was significantly correlated with the membrane PKC ζ level analyzed with PKC ζ-specific antibody, which is well in agreement with the earlier observations that ceramide produced by acid SMase activates PKC ζ.[31,32] The significant correlation between neutral SMase activity and the 40 & 43 kDa fraction of cytosolic PKC ζ/λ is a new observation and may suggest an involvement of neutral SMase in down-regulation of PKC ζ/λ.

In conclusion, the present study demonstrates that a high-beef diet is capable of modulating colonic PKC isozyme expression and intracellular localization. These results are noteworthy because a number of experimental studies have shown that the neoplastic process in the colon involves epigenetic changes in PKC activity and isozyme expression, and that some dietary nutrients and pharmacological agents affect the neoplastic process partly through PKC. Furthermore, there is considerable epidemiological evidence suggesting an association with high-red meat intake and an increased colon cancer risk, but relatively little experimental evidence on the mechanisms mediating this effect.

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