Dietary conjugated linoleic acid (CLA) induces apoptosis of colonic mucosa in 1,2-dimethylhydrazine-treated rats: a possible mechanism of the anticarcinogenic effect by CLA

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One of the objectives of the present study was to investigate whether 1 % conjugated linoleic acid (CLA) in the diet reduced tumour incidence in the colon of 1,2-dimethylhydrazine (DMH)-treated rats. Colon cancer was induced by injecting 6-week-old, male, Sprague–Dawley rats with 15 mg/kg DMH twice per week for 6 weeks. They were fed either 1 % CLA or a control diet *ad libitum* for 30 weeks. Dietary CLA significantly decreased colon tumour incidence ($P<0.05$). Our second objective was to investigate whether apoptosis in the colon mucosa of DMH-treated rats was affected by the amount of dietary CLA and whether the changes in apoptosis were related to those in fatty acid-responsive biomarkers. For this purpose, rats were killed after being fed a diet containing 0 %, 0.5 %, 1 % or 1.5 % CLA for 14 weeks. CLA was undetected in the mucosa of rats fed the 0 % CLA diet and increased to 5.9 mg/g phospholipid in rats fed the 0.5 % diet. The apoptotic index estimated by the terminal deoxynucleotidyl transferase-mediated dUTP nick and labelling technique was increased by 251 % and the 1,2-diacylglycerol content was decreased by 57 % in rats fed 0.5 % CLA. No further changes in these variables were observed when CLA in the diet was raised to 1.0 % or 1.5 %. However, dietary CLA decreased mucosal levels of prostaglandin E_2, thromboxane B_2 and arachidonic acid in a dose-dependent manner. The present data indicate that dietary CLA can inhibit DMH-induced colon carcinogenesis by mechanisms probably involving increased apoptosis.

Conjugated linoleic acid: Colon cancer: Prostaglandin E_2: Thromboxane B_2: 1,2-diacylglycerol: Apoptosis

Conjugated linoleic acid (CLA) is found in natural sources, such as the fat of milk and meat of ruminant animals. CLA is a collective term used to denote one or more positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12-18:2). In animal studies, CLA has proved to be a potent anticarcinogen (Parodi, 1997) reducing the incidence of chemically induced epidermal, mammary and forestomach tumours in mice and aberrant crypt foci in the rat colon. Since colon cancer is one of the most frequent malignant diseases in the Western world, it is important to study the mechanisms by which dietary CLA inhibits colon carcinogenesis.

Increased expression of cyclo-oxygenase (COX) and overproduction of prostaglandins (PG) have been indicated in the development and progression of colorectal cancer.

Colon cancer is prevented or attenuated in mice or humans treated with COX inhibitors (Oshima *et al.* 1996; Williams *et al.* 1997; Prescott & Fitzpatrick, 2000). As CLA and linoleic acid are likely to share the same enzyme system for chain elongation and desaturation, CLA feeding decreases the amount of linoleic acid metabolites including 18:3n-6, 20:3n-6 and 20:4n-6 in mammary tissues (Banni *et al.* 1999). Decreased arachidonate, a substrate for COX and lipoxygenase, would result in decreased eicosanoid synthesis. Indeed, PGE_2 and 6-keto-$PGF_{1\alpha}$ levels have been reported to be reduced in the colon mucosa of CLA-fed rats (Xu & Dashwood, 1999). *In vitro* studies have also shown that CLA inhibits PGE_2 synthesis from arachidonate in murine keratinocytes (Liu & Belury, 1998). The eicosanoid receptors have been shown to control the release

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of second messengers such as cyclic AMP, 1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate, which are important for cell proliferation, differentiation and apoptosis (Marks *et al.* 2000). Overexpression of COX-2 inhibits apoptosis (Tsujii & DuBois, 1995) and apoptosis appears to mediate the chemopreventive effects of non-steroidal anti-inflammatory drugs (NSAID; Samaha *et al.* 1997). Therefore, it can be hypothesized that dietary CLA inhibits colon carcinogenesis by inducing apoptosis through mechanisms involving inhibition of eicosanoid synthesis.

The present study investigated whether dietary CLA inhibited colon tumorigenesis induced by 1,2-dimethylhydrazine (DMH) in rats. In addition, we investigated whether CLA stimulated apoptosis in the colon mucosa and whether the changes in apoptosis stimulated by CLA were related to other fatty acid-responsive biomarkers such as PGE_2, thromboxane $(TX)B_2$ and DAG.

Table 1. The fatty acid composition of diets used in experiment 1

Fatty acid	0·0 % CLA (g/100 g diet)	1·0 % CLA (g/100 g diet)
C14:0	0·30	0·26
C14:1	0·11	0·12
C16:0	2·53	2·24
C16:1	0·39	0·34
C17:1	0·10	0·08
C18:0	1·77	1·56
C18:1	4·70	4·31
C18:2*n*-6	1·69	1·74
C18:3*n*-3	0·02	0·02
CLA*	0·00	1·00
C20:0	0·11	0·10
Unknown	0·22	0·22
Total	11·94	11·99

CLA, conjugated linoeic acid.
* The isomeric composition of the CLA was 50·7 % *trans*-10,*cis*-12, 47·2 % *cis*-9,*trans*-11 CLA, 1·2 % *trans*, *trans*-CLA and 0·9 % other CLA isomers.

Methods

Experiment 1

All experimental procedures were conducted in compliance with the revised National Institutes of Health Guide for the Care and Use of Laboratory Animals. Sixty male Sprague–Dawley rats, 6 weeks of age, were purchased from Deahan Animal Experimental Center (Choong-buk, Korea). Upon arrival, they were individually housed in hanging stainless-steel cages on a 12 : 12 h light–dark cycle and given Purina rat chow (Ralston-Purina, St Louis, MO, USA) and tap water *ad libitum*. After 7 d of acclimatization, all rats were injected intramuscularly with DMH (Aldrich Chemical Co., Milwaukee, WI, USA) at a dose of 15 mg/kg body weight twice per week for 6 weeks to deliver a total dose of 180 mg/kg. At the same time, the rats were randomly divided into two dietary groups and fed either the control or the experimental diet *ad libitum*. The composition of the control diet was as follows (g/kg): maize starch, 565; casein, 220; L-methionine, 3; cellulose, 40; beef tallow, 96·4; maize oil, 23·6; American Institute of Nutrition (AIN): AIN-76 mineral mix, 40; modified AIN-76 vitamin mix, 10; choline bitartrate, 2. The experimental diet contained the same dietary ingredients as the control diet, except that this diet contained 12·5 g CLA-rich oil in place of a portion of the beef tallow. CLA-rich oil was synthesized from commercial safflower oil by alkali isomerization as previously described (Kim *et al.* 2000) and the fatty acid profile of the oil was determined by GC as described further. The CLA-rich oil contained 80 % CLA isomers, and the isomeric composition of the CLA was 50·7 % *trans*-10,*cis*-12, 47·2 % *cis*-9,-*trans*-11 CLA, 1·2 % *trans*, *trans*-CLA and 0·9 % other CLA isomers. The fatty acid composition of diets is shown in Table 1. The amount of CLA-rich oil was calculated to formulate a diet containing 1 % (w/w) pure CLA. Food intakes and body weights were determined weekly. The animals were killed 30 weeks after the initiation of feeding of the experimental diets, and the colon was removed, opened, washed in PBS, fixed in 10 % buffered formalin and examined under a dissecting microscope for neoplasm.

Experiment 2

Animals, diet and study protocols. After acclimatization, rats received intramuscular injections of DMH as described earlier. Immediately before the initiation of the DMH injections, the animals were randomly divided into four groups and fed a diet containing 0 %, 0·5 %, 1 %, or 1·5 % (w/w) CLA *ad libitum* (Table 2). They were killed 14 weeks after the initiation of the DMH injections and feeding of experimental diets. The colon was removed and 1 cm segments of the distal colon were rinsed in PBS and fixed in 10 % buffered formalin for the estimation of apoptotic cells. The remaining mucosa was scraped from the underlying tissue with a glass slide for the determination of PGE_2, TXB_2, DAG and fatty acid composition of phospholipids.

Determination of fatty acid profile of phospholipids and 1,2-diacylglycerol levels in the colonic mucosa. Mucosal lipids were extracted according to Bligh & Dyer (1959) and phospholipids were isolated by TLC by the method of Duncan *et al.* (1993). Fatty acid methyl esters were prepared (Lepage & Roy, 1984) and fatty acid compositions were analysed by GC using a Hewlett-Packard 5890 II series chromatograph equipped with flame-ionization detector (Hewlett-Packard, Downers Grove, IL, USA) and a fused silica capillary column (SP-2330, 0·25 mm ID ×60 m; Supelco, Bellefonte, PA, USA). The initial and final temperatures were 170°C and 245°C respectively, with a temperature programme 2°C rise/min after a 4 min delay at 170°C. For the determination of mucosal levels of DAG, total lipids were extracted from the mucosa as described earlier. DAG was separated on a silica gel TLC plate according to the method described by Duncan *et al.* (1993), scraped from the plate, and quantified as described by Fletcher (1968) using DAG as a standard.

Apoptosis. The fixed tissues were embedded in paraffin, and paraffin sections (4 μm thickness) were mounted on albumin-coated glass slides and deparaffinized. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) was carried out utilizing an *in situ* cell death detection kit (AP; Roche Molecular Biochemical, Mannheim, Germany) according to Gavrieli *et al.* (1992).

Table 2. Composition of the diets used in experiment 2 (g/100 g). Percentages are of pure conjugated linoleic acid (CLA) in the diet

	0 % CLA	0·5 % CLA	1·0 % CLA	1·5 % CLA
Maize starch	54·60	54·60	54·60	54·60
Casein	21·60	21·60	21·60	21·60
L-methionine	0·30	0·30	0·30	0·30
Cellulose	3·70	3·70	3·70	3·70
Fat sources				
Beef tallow	12·0	11·23	10·47	9·70
Maize oil*	2·50	2·55	2·59	2·63
CLA-rich oil†	—	0·72	1·44	2·15
AIN-76 mineral mix	4·00	4·00	4·00	4·00
AIN-76 vitamin mix	1·00	1·00	1·00	1·00
Choline bitartrate	0·30	0·30	0·30	0·30

* The amount of maize oil was added to supply 1·79 % linoleic acid in each diet.
† CLA-rich oil contained 70 % CLA isomers, and the isomeric composition of the CLA was 56 % *trans*-10,*cis*-12 and 39 % *cis*-9,*trans*-11-CLA.

Samples were counterstained with methyl green prior to analysis by light microscopy. Ten vertically oriented crypt and villus columns were evaluated per animal. The numbers of apoptotic cells and total cells were counted. The apoptotic index was calculated as the number of nuclei divided by the total number of nuclei per crypt column.

Determination of prostaglandin E_2 and thromboxane B_2 levels. Mucosa was homogenized in 0·05 M-Tris buffer, pH 8·0, containing 0·25 M-sucrose and 1 mM EDTA and centrifuged at 4000 g for 15 min, and the supernatant was saved as described previously (Vanderhoof *et al.* 1988). PGE_2 and TXB_2 were extracted by a solid-phase C-18 cartridge (Alltech Associates, Inc., Deerfield, IL, USA), and the amounts of PGE_2 and TXB_2 were determined using the enzyme immunoassay kits according to the manufacturer's instructions (Cayman, Ann Arbor, MI, USA).

Statistical analysis. Data were analysed using the Statistical Analysis Systems statistical software package version 6.12 (SAS Institute, Cary, NC, USA). Tumour incidences at the final time point were compared by χ^2 test and the total tumour yield between the control and the CLA-treated groups was compared by frequency distribution analysis. Most data were expressed as means and standard deviations. One-way ANOVA, followed by Duncan's multiple range test, was used to analyse the data from experiment 2. The level of significance was set at $P < 0·05$.

Results

Experiment 1

To examine whether feeding a diet containing 1 % CLA reduced the incidence of colon tumours, tumours were induced in rats by injecting DMH at the dose of 15 mg/kg twice per week for 6 weeks. Feeding rats with the diet containing 1 % CLA had no effect on the pattern of food intake or weight gain during the 30 weeks of the experimental period compared to the pattern in control rats (data not shown). As shown in Table 3, the incidence of tumours was significantly lower in rats fed the 1 % CLA diet compared to control rats ($P < 0·05$).

Experiment 2

Since we observed that the diet containing 1 % CLA significantly reduced the incidence of tumours, the following experiments were performed to examine the mechanisms by which CLA inhibited colon carcinogenesis. The rats were injected with DMH as in experiment 1, but were fed diets containing different levels of CLA (0 %, 0·5 %, 1 %, or 1·5 %) for 14 weeks. In this part of the study, examination of the colonic mucosa was carried out before the development of grossly visible neoplasia. There were no differences in growth characteristics and food intake patterns of rats during the 14 week period among the four dietary groups (data not shown).

The rate of tumour growth is dependent upon a balance between the rates of cell proliferation and apoptosis. To determine whether apoptotic rates of colonic mucosal cells were increased by dietary CLA, apoptosis was estimated *in situ* by the TUNEL technique. As shown in Table 4, the apoptotic index was significantly increased in rats fed the 0·5 % CLA diet compared to control rats fed no CLA ($P < 0·05$). However, there was no significant difference in the apoptotic index among the three groups fed different levels of CLA.

To examine whether changes in apoptosis observed in CLA-fed rats were related to mucosal PG and TX levels, PGE_2 and TXB_2 were quantified by enzyme immunoassays. Dietary CLA decreased levels of PGE_2 and TXB_2 in the colonic mucosa in a dose-dependent manner. However, neither the difference in these parameters between the 0·5 %

Table 3. Effects of conjugated linoleic acid (CLA) on 1,2-dimethylhydrazine (DMH)-induced colon tumours in rats. Autopsy was performed after feeding DMH-treated rats with the indicated diet for 30 weeks

Diet	Tumour incidence†	Total tumour number	Mean tumour number per rat
Control	12/30	18	0·60
CLA	7/30*	10*	0·33*

† Number of tumour-bearing rats/total number of rats in each group.
Significantly different from control: * $P < 0·05$.

Table 4. Effect of conjugated linoleic acid (CLA) on apoptotic index in the colonic mucosa of rats treated with 1,2-dimethylhydrazine

(Mean values and standard deviations)

Dietary group (% CLA)	Number of rats	Apoptotic index	
		Mean	SD
0·0	5	0·1985[b]	0·0664
0·5	6	0·4983[a]	0·1961
1·0	5	0·4577[a]	0·1486
1·5	5	0·5159[a]	0·0492

[a,b] Values with unlike superscript letters were significantly different ($P<0.05$).

Table 5. Effect of conjugated linoleic acid (CLA) on mucosal prostaglandin (PG)E_2 and thromboxane (TX)B_2 levels (ng/mg tissue) in the colon of rats treated with 1,2-dimethylhydrazine

(Mean values and standard deviations)

Dietary group (% CLA)	Number of rats	PGE_2		TXB_2	
		Mean	SD	Mean	SD
0	7	21·06[a]	1·11	6·91[a]	1·11
0·5	9	16·04[b]	4·19	5·70[b]	1·01
1·0	7	14·14[bc]	2·80	4·41[bc]	0·81
1·5	8	10·47[c]	5·28	3·78[c]	0·56

[a,b,c] Values with unlike superscript letters were significantly different ($P<0.05$).

Table 6. Effect of conjugated linoleic acid (CLA) on the fatty acid composition of mucosal phospholipids of the colon in rats treated with 1,2-dimethylhydrazine

(Mean and standard deviations for values expressed as milligrams fatty acid per gram phospholipid)

Dietary group (% CLA)	Number of rats	CLA		AA	
		Mean	SD	Mean	SD
0	6	ND	—	5·13[a]	2·02
0·5	6	5·88[a]	5·60	3·65[ab]	1·27
1·0	6	7·68[a]	6·58	1·66[b]	1·91
1·5	6	6·04[a]	3·07	1·80[b]	1·70

AA, arachidonic acid; ND, not detected.
[a,b] Values with unlike superscript letters were significantly different ($P<0.05$).

Table 7. Effect of conjugated linoleic acid (CLA) on 1,2-diacylglycerol (DAG) levels of the colonic mucosa in rats treated with 1,2-dimethylhydrazine

(Mean and standard deviations for values expressed as nanograms per milligram tissue)

Dietary group (% CLA)	Number of rats	DAG	
		Mean	SD
0	6	0·23[a]	0·12
0·5	6	0·10[b]	0·04
1·0	8	0·14[b]	0·06
1·5	8	0·12[b]	0·04

[a,b] Values with unlike superscript letters were significantly different ($P<0.05$).

and 1·0 % CLA groups nor the difference between the 1·0 % and 1·5 % groups was statistically significant (Table 5).

To examine whether the decreased PGE_2 and TXB_2 levels were related to fatty acid profiles of cell membranes, the mucosal phospholipids were separated. As shown in Table 6, CLA was not detectable when rats were fed the 0 % CLA diet. When rats were fed the diet containing 0·5 % CLA, the levels of CLA reached 5·88 mg/g mucosal phospholipids. However, increases in dietary CLA over 0·5 % did not further increase CLA content of mucosal phospholipids in the colon of DMH-injected rats. By contrast, arachidonic acid content decreased significantly in the 1·0 % and 1·5 % CLA groups compared to the 0·0 % CLA group ($P<0.05$). CLA feeding did not alter the proportion of any other fatty acid (data not shown).

Another possible means by which CLA could increase apoptosis in the colonic mucosa would be to decrease DAG levels in mucosal cells. Mucosal DAG levels were significantly lower in the 0·5 % CLA group than those in the 0 % CLA group ($P<0.05$). However, there were no differences in DAG levels among the groups fed different levels of CLA (Table 7).

Discussion

The present study has clearly shown that dietary CLA at 1 % concentration inhibits DMH-induced colon carcinogenesis in rats. It has been reported that CLA administered by gavage at a level of 0·5 % of diet (w/w) reduced formation of heterocyclic amine-induced aberrant foci in the colon of Fisher 344 rats (Liew *et al.* 1995; Xu & Dashwood, 1999). In addition to colon cancers, studies with rat mammary cancers induced by 7,12-dimethylbenz[α]anthracene (Ip *et al.* 1996) showed that the inhibitory effect of CLA reached a maximum at 1 % regardless of the levels of fat in the diet. In studies with transplantable murine mammary tumours grown in mice fed 20 % fat diets, Hubbard *et al.* (2000) observed that the concentration of CLA required for inhibition of mammary tumour metastasis was as little as 0·1 % of the diet compared with diets containing no CLA. It was also reported that CLA decreased local growth and the metastatic properties of the DU-145 human prostate carcinoma cell line in mice suffering from severe combined immunodeficiency (Cesano *et al.* 1998). Mice receiving a diet supplemented with 1 % CLA not only displayed smaller local tumours than mice receiving the regular diet but also had a drastic reduction in lung metastases. Results of all these studies indicate that concentrations of CLA ≤ 1 % are sufficient for producing significant cancer protection in rodent models.

The anti-carcinogenic effect of CLA shown in the present study may be isomer specific. However, the synthetically prepared CLA used in the present study contained eight different isomers, with two particular isomers, *trans*-10,-*cis*-12 and *cis*-9,*trans*-11, accounting for about 97 % of the total. We have *in vitro* data indicating that CLA inhibits proliferation of the human colon cancer cell line Caco-2, which is owing to the effect of the *trans*-10,*cis*-12 isomer (Kim *et al.* 2001). It remains to be determined whether this isomer is the biologically active species for colon cancer prevention *in vivo*.

Cancer is frequently described as a disorder of the balance between cell proliferation and cell death. We observed that dietary CLA increased the apoptotic index in the colon mucosa, indicating that CLA inhibits DMH-induced colon carcinogenesis by inducing apoptosis. Recently, Ip *et al.* (2000) reported that CLA induced apoptosis and reduced the expression of bcl-2 in premalignant lesions of the rat mammary gland but not in normal mammary gland. However, we cannot rule out the possibility that CLA induces proliferation arrest of mucosal cells, as the present experiments did not determine whether inhibition of colon cancer by CLA was due to decreased cellular proliferation. *In vitro* studies have shown that CLA inhibits the proliferation of MCF-7 human breast cancer cells. Cell cycle analysis indicated that more of the MCF-7 cells remained in the G_0/G_1 cycle when the cells were cultured with CLA (Durgam & Fernandes, 1997).

Phosphatidylinositol-4,5-bisphosphate is part of a second messenger system that transduces signals of many hormones. The membrane-bound phospholipase C catalyses the hydrolysis of phosphatidylinositol-4,5-bisphosphate at its glycerol–phospho bond, yielding inositol-1,4,5-trisphosphate and DAG. The non-polar DAG product of phospolipase C action is a lipid-soluble second messenger remaining in the plasma membrane, where it activates protein kinase C to phosphorylate and thereby modulate the activities of several cellular proteins. Protein kinase C has been proposed to play an important role in the aetiology of colon cancer by regulating gene expression and a variety of cellular functions, including proliferation, differentiation, tumorigenesis and apoptosis (Nishizuka, 1986, 1988; Newton, 1997). It has been reported that DAG was down-regulated during apoptotic cell death (Voziyan *et al.* 1995; Chen *et al.* 2000) and exogenous DAG inhibited the apoptosis triggered by farnesol, ceramide and daunorubicin (Jarvis *et al.* 1994*a*; Voziyan *et al.* 1995; Mansat *et al.* 1997), suggesting DAG as an important antiapoptotic and survival signal. In the present study, changes in DAG levels (Table 7) were negatively related to those in the apoptotic index in the colon mucosa of CLA-fed rats (Table 4). These results are consistent with the hypothesis that DAG is a positive intracellular mitogenic agent (Jarvis *et al.* 1994*b*; Flores *et al.* 2000).

Previous studies using both genetic and pharmacological approaches have established that COX-2 plays a role in the development of colorectal cancer (Gupta & DuBois, 1998; Williams *et al.* 1999). NSAID such as sulindac cause regression of precancerous colonic lesions in animals (Moorghen *et al.* 1988) and human subjects (Takayama *et al.* 1998). NSAID exert their chemopreventive effects by inhibiting COX-2-dependent (Prescott & White, 1996) or COX-2-independent pathways that trigger apoptosis (Piazza *et al.* 1995; Shiff *et al.* 1995). In the present study, CLA lowered both PGE_2 and TXB_2 levels in a dose-dependent manner in the colonic mucosa of DMH-injected rats (Table 5). These results are consistent with the results of Xu & Dashwood (1999) showing that PGE_2 and 6-keto-$PGF_{1\alpha}$ were reduced in the colonic mucosa of CLA-fed rats. Similar decreases in PGE_2 synthesis by CLA were observed in the epidermis of mice treated topically with the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate

(Kavanaugh *et al.* 1999). Since CLA reduced PGE_2 and TXB_2, a tenable hypothesis is that CLA and NSAID share a common path of action in promoting the apoptotic process.

Dietary CLA reduced the arachidonate content of phospholipids in the colonic mucosa of DMH-treated rats (Table 6). These decreases in arachidonate may have caused the decreased PGE_2 and TXB_2 observed in these animals. Using *in vitro* mouse keratinocyte cultures, Liu & Belury (1998) observed that CLA decreased cellular AA content and arachidonate-derived PGE_2 synthesis induced by 12-*O*-tetradecanoylphorbol-13-acetate. Sébédio *et al.* (1997) have shown that rats fed a fat-free diet for 2 weeks metabolized a CLA mixture to C20:3Δ8,12,14, C20:4Δ5,8,12,14, and C20:4Δ5,8,11,13 with a higher quantity of C20:4Δ5,8,12,14 than C20:4Δ5,8,11,13. C20:4Δ5,8,12,14 and C20:4Δ5,8,11,13 must arise from the elongation and desaturation of 18:2Δ10,12 and 18:2Δ9,11 respectively. As the present study did not measure these products, we can only speculate that CLA was desaturated and elongated to the 20 C conjugated fatty acids that decreased arachidonate-derived PGE_2 and TXB_2 in the colonic mucosa of our CLA-fed animals. Mechanisms by which dietary CLA modulates arachidonic acid metabolism needs to be investigated further.

In summary, dietary CLA reduced the incidence of tumours, and induced apoptosis in the colonic mucosa of DMH-treated rats, suggesting that CLA inhibits colon carcinogenesis, at least in part, by increasing apoptosis. Levels of regulators of cell responses, PGE_2, TXB_2 and DAG, decreased in the colonic mucosa of CLA-fed rats. These results suggest that the increased apoptosis by dietary CLA may be, at least in part, attributable to changes in arachidonic acid metabolism in CLA-fed rats. The precise signalling event involved in CLA-induced apoptosis in colon mucosal cells remains to be determined further.

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