Effects of dietary fat and 1,2-dimethylhydrazine on microsomal lipid peroxidation

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An increased intake of polyunsaturated fatty acids, especially those of the omega-3 series, has shown beneficial effects in prevention of chronic diseases, including cancer. It is not known if at high levels, incorporation of these fatty acids into membrane phospholipids will cause adverse effects due to increased tissue susceptibility to peroxidation. This study was designed to determine whether diets high in polyunsaturated fatty acids would increase liver and colon susceptibility to peroxidation. Secondly, the study examined the effect of diet and the colon carcinogen, 1,2-dimethylhydrazine, on potential peroxidation in these tissues. Rats were fed diets of varied fatty acid composition and effects were compared over time and as the result of injection of 1,2-dimethylhydrazine. Test diets consisted of a low fat corn oil diet or high fat diets of either corn oil, beef tallow, or menhaden oil. Fatty acid composition of colon microsomes was determined. Lipid peroxidation products were measured as thiobarbituric acid-reacting substances in liver and colon microsomes before and after induction with iron/ADP/ascorbate. Content of polyunsaturated fatty acids in colon microsomes was shown to vary according to diet. Induced peroxidation of liver microsomes was highest in the menhaden oil group but only in young animals. Older animals showed greater levels of induced peroxidation in the liver and less effect of diet. Dimethylhydrazine increased inducible peroxidation in the liver without a clear pattern of difference by diet. Tissue differences were also apparent. Colon microsomes showed resistance to peroxide induction by iron/ADP/ascorbate and no difference in peroxide content as the result of DMH. Increases in lipid peroxidation do not appear to be associated with this model of colon carcinogenesis. (J. Nutr. Biochem. 5:78–83, 1994.)

Keywords: diet; lipid peroxidation; carcinogenesis; 1,2-dimethylhydrazine; polyunsaturated fatty acids

Introduction

Results from a number of studies in the area of coronary heart disease and cancer have shown beneficial effects from increasing consumption of omega-3 fatty acids.[1,2] There are currently health concerns associated with increasing total quantity of these fatty acids in the diet, however.[3] The primary reason for concern is the possibility of increased lipid peroxidation in vivo and the toxic effects this might have on tissues.[4] Over the past several years, a body of evidence has accumulated suggesting that the products of lipid peroxidation may play important physiological roles as well.[5] Lipid peroxidation reflects the inability of cellular antioxidant mechanisms to adequately protect membrane phospholipids from oxidative reactions. These oxidative reactions, as well as the lipid peroxidation products themselves, can be highly destructive to cells and have been causally related to a number of chronic diseases including some, but not all, cancers.[4]

It is known that in many tissues the fatty acid composition of membrane phospholipids has been shown to reflect the fatty acid content of the diet, especially with regard to the types of polyunsaturated fatty acids (PUFA) that become incorporated.[6] These PUFA alter many functions of the membrane[7] and can change membrane characteristics,[8] including susceptibility to peroxidation.[9] Preparations of liver microsomes have been of particular interest with regard to fatty acid composition and oxidative metabolism of carcinogens.[10] Generally, membranes that are high in PUFA are more susceptible to peroxida-

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tion and require greater antioxidant protection in response to an oxidative stress.[6] It is interesting to note, however, that in contrast to this generalization some studies have proposed that moderate amounts of omega-3 fatty acids within membranes may actually interfere with the formation of lipid peroxides by competitively inhibiting the oxidation of arachidonic acid by cyclooxygenase and lipoxygenase, thus protecting antioxidants from overconsumption.[11] And, in studies of tumors there are conflicting results with regard to correlation of lipid peroxidation products and unsaturated fatty acid content of membranes.[12] Thus, the first objective of this study was designed to test the hypothesis that diets high in PUFA would create microsomal membranes with a greater potential for peroxidation.

Free radicals have been implicated in both the initiation and promotion stages of cancer.[13] Many studies have focused on the possible role of alterations in lipid peroxidation in the multi-stage formation of tumors.[4] Diets high in omega-3 fatty acids have been shown to promote fewer tumors than other high fat diets in rodent models of colon carcinogenesis.[2] In addition, other analyses have identified animal fat, generally containing a higher percentage of saturated fatty acids, as the most strongly correlated factor for colon cancer in humans.[14,15] The carcinogen, 1,2-dimethylhydrazine (DMH), specifically causes colon tumors in experimental animals.[16] DMH is metabolized in the liver, and possibly in the colon itself, to a methyl radical.[17,18] Colon mucosa has been shown to be very resistant to peroxidation by this laboratory[19] and others,[20] making the role of lipid peroxidation in carcinogenesis unknown in this tissue. Therefore, the second objective of this study examined interactions between diet and DMH in liver and colon in an effort to determine if lipid peroxidation of membranes may result from exposure to this carcinogen.

Materials and methods

Animals and diets

To study the effects of feeding diets varied in lipid source on hepatic microsomal lipid peroxidation, 180 weanling male Sprague-Dawley rats (Crl:CD® BR, VAF/Plus) purchased from Charles River (Kingston, NY USA) were randomly assigned to four different dietary treatment groups. All diets were formulated by and purchased from Dyets (Bethlehem, PA USA). Test diets included the basal diet (BD), which was an AIN-76A–based[21,22] low fat diet containing 5% corn oil, a modified high fat diet containing 20% corn oil (CO), a modified high fat diet containing 19% menhaden oil and 1% corn oil (MO), and a modified high fat diet containing 19% beef tallow and 1% corn oil (BT). All lipids contained 0.02% tert-butylhydroxyquinone (TBHQ) to prevent oxidation. Details of the diet composition have been published previously.[23] The vitamin, mineral, and fiber content of the high fat diets was adjusted so that they would be the same as the low fat diet on a per calorie basis. However, the tocopherol content of the diets varied due to the use of unstripped corn oil. *Table 1* summaries the antioxidant vitamin and mineral composition of the diets.

Animals were housed individually in suspended stainless-steel mesh cages and maintained at 22° C, with a 12-hr light:

Table 1 Antioxidant vitamin and mineral composition of diets*

	Diets			
	BD	MO	CO	BT
Vitamin E (IU/kg diet)†	50.0	60.0	60.0	60.0
RRR-α-tocopherol (mg/kg diet)‡	7.5	1.5	30.0	4.0
Selenium (mg/kg diet)§	0.10	0.12	0.12	0.12

*Summarized from Kuratko and Pence.[23]
†Provided by AIN-76A vitamin mix according to Bieri.[22]
‡Calculated from lipid analysis provided by Dyets.
§Provided by AIN-76 mineral mix.[21]
BD, 5% corn oil; MO, 19% menhaden and 1% corn oil; CO, 20% corn oil; BT, 19% beef tallow and 1% corn oil.

dark cycle. All animals were cared for in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, and institutional approval was granted prior to experimentation. The animals were euthanized with CO_2 and livers were collected after 2 weeks, 1, 6, and 9 months of feeding the diets.

To study the interactive effects of diet and DMH, 160 weanling male Sprague-Dawley rats were randomly assigned to one of the four dietary treatment groups for 5 weeks. Animals from each dietary group were given one, two, or three intraperitoneal injections of 1,2-dimethylhydrazine dihydrochloride (20 mg/kg body weight) or vehicle control (1 mmol/L EDTA). More than one dose of DMH was given because DMH is usually given as a multi-dose regimen.[24] Animals were killed 3 hours after injection, livers and colons were excised, and microsomal preparations were made for immediate storage at −90° C until analysis. The 3-hour time post DMH injection was chosen based on previous studies that examined colon antioxidant activity and lipid peroxidative damage after DMH administration.[25]

Microsome preparation

Livers and colons were excised from the male Sprague-Dawley rats that were maintained on the test diets for specified time intervals. The livers were rinsed thoroughly in ice-cold 1.15% KCl, minced, and homogenized in 0.05 mol/L Tris-HCl (pH 7.4)/1.15% KCl, followed by a 10 min 10,000*g* centrifugation. The colons were excised at the rectal and cecal junctions, slit, fecal debris removed, and the mucosa scraped with a spatula. Colon mucosal tissue was then treated similarly to liver. The supernatant from this fraction was centrifuged at 105,000*g* for 60 min. The microsomal pellet was resuspended in Tris/KCl/0.25 mol/L sucrose and stored at −90° C until used in the assays. Protein concentration was determined by the method of Bradford,[26] using the Bio-Rad reagent (Bio-Rad Laboratories, Richmond, CA USA).

Microsomal peroxidation

Estimates of lipid peroxidation were determined by measuring thiobarbituric acid reacting substances (TBARS) by modifications of the procedures of Asakawa[27] and Ohkawa.[28] The reaction mix contained 200 μL microsomal suspension, 400 μL buffer (50 mmol/L $NaKPO_4$ buffer with 0.1 mmol/L EDTA, pH 7.0), 600 μL 10% trichloroacetic acid, and 1.0 mL 0.5% 2-thiobarbituric acid. After heating the reaction mix in boiling water for 45 min, tubes were cooled, centrifuged, and the absorbance of 1.0 mL of supernatant was determined at 535 nm against a standard curve of 1,1,3,3-tetramethoxypropane.

Microsomal peroxidation was induced with the addition of 0.6 mmol/L ascorbic acid, 5 μmol/L $FeCl_3$, and 50 μmol/L ADP in 0.05 mol/L Tris-HCl (pH 7.4), followed by incubation at 37° C for 1 hour, according to Ursini et al.[29] The addition of butylated hydroxytoluene (BHT) to a concentration of 0.01% was used to stop the induction.

Fatty acid analysis

Colon microsomes prepared as described above were used for fatty acid analysis by gas chromatography. Microsomal preparations were extracted using methanol and chloroform[30] for analysis of total lipids. Fatty acids of the lipid extracts were prepared by transmethylation and composition analyses of the fatty acid methyl esters were then determined on a Varian 3300 gas chromatograph (Varian, Palo Alto, CA USA) equipped with a flame ionization detector and interfaced with a Hewlett-Packard 3390A integrator (Palo Alto, CA USA). Peaks were identified based on retention times relative to authentic fatty acid methyl esters obtained from Matreya (Pleasant Gap, PA USA).

Reagents used

All reagents and chemicals were purchased from Sigma Chemical Company (St. Louis, MO USA) unless otherwise specified.

Statistical analysis

SAS statistical software[31] was used for statistical analysis (SAS Institute, Cary, NC USA). Analysis of variance procedure was applied using the general linear model of SAS and significance set at $P < 0.05$. Duncan's multiple range test was used to separate treatment means when the initial analysis of variance indicated a significant difference.

Results

Animal growth

Corn oil used in the experimental diets of this study contained 60% linoleic acid (fatty acid composition provided by Dyets). The essential fatty acid requirement for the laboratory rat is estimated to be 0.6% of the diet.[32] Therefore, all diets used in this study provided adequate levels of essential fatty acids. Body weights of animals in all groups increased at a normal rate and are shown in *Figure 1.*

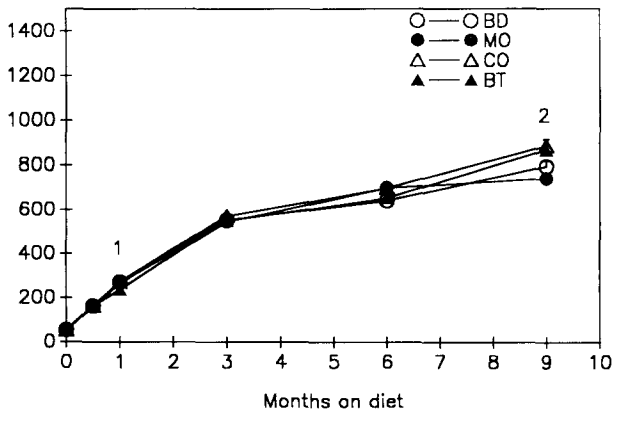

Figure 1 Body weights of rats fed test diets. [1]BT differs from BD, MO, and CO ($P < 0.05$). [2]BT and MO differ from BD and CO ($P < 0.05$).

Fatty acid composition of colon microsomes

The diets used in this experiment modified the fatty acid composition of colon mucosal microsomes as shown in *Table 2.* The fatty acid pattern reflected the dietary lipid sources with some modifications. The content of membrane saturated fatty acids did not vary widely, with the BD, MO, CO, and BT groups containing 33.3%, 36.2%, 30.6%, and 31.9%, respectively. Monounsaturated fats were highest, at 42.0% in the BT animals, with the BD, MO, and CO groups containing 28.4, 27.4, and 22.1%, respectively. MO-fed animals showed lower levels of arachidonic acid (20:4, n-6) and increased levels of longer chain omega-3 fatty acids (20:5, 22:5, and 22:6) as compared with the other diets. The corn oil-containing diets (BD and CO) showed a greater incorporation of linoleic acid (18:2, n-6) than the MO or BT diets. Incorporation of linoleic acid appeared to be dose related, as the CO-fed animals showed greater incorporation than the BD-fed animals. The double bond index, which was calculated as the percent composition of each fatty acid multiplied by the number of double bonds in that acid divided by 100, is also reported in *Table 2.* The MO diet created membranes with the highest double bond index.

Effect of diet treatment alone on liver microsomes

The effects of feeding the experimental diets for a period of up to 9 months are shown in *Table 3.* Only a small amount of non-induced lipid peroxidation of liver microsomes was detectable in untreated rats, with no consistent, overall differences between diets. Induced lipid peroxidation was highest in the MO-fed group in younger rats. Inducible lipid peroxidation increased with age in all of the diet groups, with the levels determined at the 9-month time point being significantly greater than the 2-week time point in all diets.

Effects of diet treatment with DMH on liver microsomes

The effects of DMH treatment on liver microsomal peroxidation are shown in *Table 4.* DMH increased inducible peroxidation in all dietary groups with no clear pattern of differences by diet. There was greater induction in MO-fed rats treated with EDTA. There was no apparent cumulative effect of two DMH injections on inducible peroxidation and, in fact, two treatments of DMH resulted in less induced peroxidation than one treatment.

Lipid peroxidation in colon mucosal microsomes

Non-induced lipid peroxidation was low and many times undetectable in colons of untreated (data not shown) and EDTA-treated rats *(Table 5).* BT-fed animals showed the lowest levels of non-induced lipid peroxidation. Peroxidation in the colon microsomes was not inducible by Fe/ascorbate/ADP. Neither inducible nor non-inducible peroxidation changed with DMH injection.

Table 2 Fatty acid composition of colon mucosal microsomes*

Fatty acid	Diet†			
	BD	MO	CO	BT
14:0	1.3 ± 0.1^{c}	3.1 ± 0.2^{a}	1.2 ± 0.1^{c}	2.0 ± 0.2^{b}
unknown‡	2.5 ± 0.2	2.4 ± 0.3	1.7 ± 0.3	1.9 ± 0.3
16:0	22.8 ± 0.9	23.1 ± 0.8	22.1 ± 0.7	19.2 ± 1.7
16:1(n-7)	4.1 ± 0.8^{ab}	5.7 ± 0.7^{a}	2.2 ± 0.5^{c}	3.0 ± 0.4^{bc}
unknown§	3.8 ± 1.1	3.0 ± 0.8	2.8 ± 1.3	2.0 ± 0.7
unknown¶	1.9 ± 0.2^{a}	2.2 ± 0.1^{a}	1.1 ± 0.2^{b}	2.0 ± 0.2^{a}
18:0	9.2 ± 0.8^{ab}	10.0 ± 0.9^{a}	7.3 ± 0.9^{b}	10.7 ± 0.3^{a}
18:1(n-9)	19.0 ± 1.5^{b}	15.8 ± 0.7^{b}	18.0 ± 1.0^{b}	34.1 ± 1.8^{a}
18:1(n-7)	4.8 ± 0.3^{a}	4.6 ± 0.1^{a}	1.5 ± 0.8^{b}	4.2 ± 0.5^{a}
18:2(n-6)	13.2 ± 1.5^{b}	4.1 ± 0.2^{c}	25.9 ± 3.9^{a}	4.9 ± 0.2^{c}
18:3(n-6)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
18:3(n-3)	0.2 ± 0.1^{b}	0.8 ± 0.1^{a}	0.6 ± 0.0^{a}	0.2 ± 0.1^{b}
18:4(n-3)	0.0 ± 0.0^{b}	0.6 ± 0.1^{a}	0.1 ± 0.0^{b}	0.1 ± 0.1^{b}
20:1(n-9)	0.5 ± 0.2^{b}	1.3 ± 0.1^{a}	0.4 ± 0.1^{b}	0.7 ± 0.0^{b}
20:3(n-6)	1.2 ± 0.2^{a}	0.4 ± 0.1^{b}	1.0 ± 0.3^{a}	0.9 ± 0.1^{ab}
20:4(n-6)	9.6 ± 1.7^{a}	2.5 ± 0.3^{b}	8.6 ± 2.1^{a}	9.2 ± 0.4^{a}
20:5(n-3)	0.1 ± 0.0^{b}	8.0 ± 0.3^{a}	0.1 ± 0.1^{b}	0.1 ± 0.0^{b}
22:4(n-6)	1.8 ± 0.2^{a}	0.3 ± 0.0^{b}	1.8 ± 0.5^{a}	1.3 ± 0.1^{a}
22:5(n-3)	0.1 ± 0.0^{b}	2.7 ± 0.2^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}
22:6(n-3)	0.3 ± 0.1^{b}	5.4 ± 0.5^{a}	0.3 ± 0.1^{b}	0.6 ± 0.0^{b}
others	3.5	3.9	3.0	2.6
double bond index‖	1.07 ± 0.05^{c}	1.39 ± 0.05^{a}	1.25 ± 0.04^{b}	1.03 ± 0.02^{c}

*Values are expressed as mean percent composition ± SEM of five rats after 5 weeks on diet.
†Means for an individual fatty acid with different letters are different (analysis of variance, Duncan's $P < 0.05$).
‡Retension time = 6.5 min.
§Retension time = 9.7 min.
¶Retension time = 10.9 min.
‖Sum of percent composition of each fatty acid × number of double bonds/100.
BD, 5% corn oil; MO, 19% menhaden oil and 1% corn oil; CO, 20% corn oil; BT, 19% beef tallow and 1% corn oil.

Table 3 Liver microsome peroxidation

Diet	Time on diet	Non-induced*†§	Induced*†‡§
BD	2 wk	1.587 ± 0.225^{a}	2.690 ± 0.576^{b}
MO		2.156 ± 0.314^{a}	11.426 ± 1.125^{a}
CO		1.689 ± 0.208^{a}	1.531 ± 0.188^{b}
BT		1.599 ± 0.290^{a}	1.591 ± 0.270^{b}
BD	1 mo	5.961 ± 0.748^{a}	8.129 ± 1.807^{b}
MO		4.237 ± 0.468^{b}	26.432 ± 2.209^{a}
CO		3.078 ± 0.508^{b}	9.395 ± 2.050^{b}
BT		2.840 ± 0.152^{b}	4.016 ± 0.195^{b}
BD	6 mo	1.052 ± 0.092^{b}	9.723 ± 3.527^{b}
MO		1.711 ± 0.189^{ab}	19.530 ± 1.840^{a}
CO		1.416 ± 0.155^{b}	11.749 ± 2.806^{b}
BT		2.131 ± 0.352^{a}	20.386 ± 1.310^{a}
BD	9 mo	2.237 ± 0.259^{b}	14.808 ± 2.022^{b,}¶
MO		2.727 ± 0.367^{ab}	25.152 ± 3.534^{a,}¶
CO		3.577 ± 0.184^{a}	19.315 ± 1.378^{ab,}¶
BT		2.318 ± 0.400^{b}	21.876 ± 2.089^{ab,}¶

*Values expressed as nmol MDA/mg protein.
†Mean ± SEM of five rats.
‡Peroxidation induced by the addition of $FeCl_3$/ADP/ascorbate.
§Means within the same time period with different letters are different (analysis of variance, Duncan's $P < 0.05$)
¶Differ from corresponding diet at the 2-wk time point (analysis of variance, Duncan's $P < 0.05$)
BD, 5% corn oil; MO, 19% menhaden oil and 1% corn oil; CO, 20% corn oil; BT, 19% beef tallow and 1% corn oil.

Table 4 Liver microsome peroxidation following DMH treatment

Diet	Treatment*	Non-induced†‡¶	Induced†‡§¶
BD	EDTA-1	1.607 ± 0.212	2.528 ± 0.224^{b}
MO		2.044 ± 0.200	10.153 ± 0.489^{a}
CO		1.347 ± 0.146	1.546 ± 0.146^{b}
BT		1.617 ± 0.188	2.757 ± 0.611^{b}
BD	DMH-1	2.366 ± 0.298^{b}	17.798 ± 3.825
MO		3.445 ± 0.392^{a}	23.129 ± 3.842
CO		2.751 ± 0.198^{ab}	22.544 ± 2.095
BT		3.321 ± 0.201^{a}	17.435 ± 1.301
BD	EDTA-2	3.543 ± 0.455	6.972 ± 3.093^{ab}
MO		3.454 ± 0.379	11.719 ± 0.750^{a}
CO		2.818 ± 0.245	7.942 ± 1.431^{ab}
BT		2.305 ± 0.219	3.059 ± 0.452^{b}
BD	DMH-2	3.342 ± 0.182	11.875 ± 1.211^{ab}
MO		3.642 ± 0.111	15.666 ± 1.215^{a}
CO		3.612 ± 0.476	10.585 ± 1.474^{b}
BT		3.708 ± 0.291	14.125 ± 1.590^{ab}

*After 5 weeks on test diets, EDTA-1 = 1 dose vehicle, DMH-1 = 1 dose DMH at 20 mg/kg body weight, EDTA-2 = 2 doses vehicle, DMH-2 = 2 doses DMH.
†Values are expressed as nmol MDA/mg protein.
‡Mean ± SEM of five rats.
§Peroxidation induced by the addition of $FeCl_3$/ADP/ascorbate.
¶Means within the same time period with different letters are different (analysis of variance, Duncan's $P < 0.05$)
BD, 5% corn oil; MO, 19% menhaden oil and 1% corn oil; CO, 20% corn oil; BT, 19% beef tallow and 1% corn oil.

Table 5 Colon microsome peroxidation following DMH treatment

Diet	Treatment*	Not induced†‡	Induced†‡§¶
BD	EDTA-3	1.38 ± 0.19[a]	1.17 ± 0.17
MO		1.32 ± 0.20[a]	1.43 ± 0.19
CO		1.54 ± 0.06[a]	1.49 ± 0.27
BT		0.82 ± 0.14[b]	1.02 ± 0.14
BD	DMH-3	1.39 ± 0.10	1.72 ± 0.24
MO		1.28 ± 0.10	1.30 ± 0.16
CO		1.57 ± 0.48	1.64 ± 0.27
BT		0.90 ± 0.08	1.12 ± 0.07

*After 5 weeks on test diets, EDTA-3 = 3 doses vehicle, DMH-3 = 3 doses DMH at 20 mg/kg body weight.
†Values are expressed as nmol MDA/mg protein.
‡Means ± SEM of 4 to 5 rats.
§Peroxidation induced by the addition of $FeCl_3$/ADP/ascorbate.
¶Means within the same time period with different letters are different (analysis of variance, Duncan's $P < 0.05$)
BD, 5% corn oil; MO, 19% menhaden oil and 1% corn oil; CO, 20% corn oil; BT, 19% beef tallow and 1% corn oil.

Discussion

In the present study, test diets were designed to reflect food sources of different fatty acid composition. The MO diet reflected a high intake of omega-3 fatty acids, the CO diet a high intake of omega-6 fatty acids, and the BT diet a high saturated and mono-unsaturated fatty acid content. The high fat diets used in this study contained 20% fat by weight, or approximately 40% of total energy, which is comparable to that consumed by the American population.[33] The data in this study appear to support our original hypothesis in part. Feeding MO-enriched diets did result in greater induced lipid peroxidation in the liver, at least in young animals. However, as the diets were fed longer periods or the animals were challenged with DMH, this was not the case.

Non-induced lipid peroxidation, measured in this study, reflects the steady-stage levels for the tissue. Although some changes occurred in non-induced lipid peroxide content of the liver as the result of diet *(Table 3)*, no detectable pattern was evident. Induced lipid peroxidation, measured in this study, is an in vitro estimation of the membrane's potential for lipid peroxidation with the addition of oxidative stress. This was accomplished by the addition of $FeCl_3$/ADP/ascorbate to the microsomal preparations. The MO-fed animals showed a greater potential for lipid peroxidation at the 2-week and 1-month time points. However, in the older animals this difference is not detectable. Many antioxidant defenses have been shown to decrease with age.[23] The results of this decrease may be reflected by the liver's increasing susceptibility to peroxidation with age, regardless of diet or membrane fatty acid composition. Tocopherol content was greatest in the diet containing 20% corn oil. The lower levels of inducible peroxidation in CO-fed animals at the 6-month time point may have resulted from the higher level of this vitamin in the diet. However, the CO group also showed age-related effects and by the 9-month time point was not different from the other high fat diets.

The administration of 1,2-dimethylhydrazine to rats is a frequently used model of experimental colon cancer.[12] DMH-induced tumors closely parallel the disease in man. It is administered in weekly doses of 20 mg/kg to produce tumors within approximately 6 months. This has allowed the investigation of pre-tumor indicators in the colon. Because the carcinogen is metabolically activated, involving the liver via a radical generated mechanism, it is especially appropriate for this study. There was an increase in induced peroxidation in the liver after DMH injections *(Table 4)*, indicating that DMH acted as an oxidative stressor by depleting antioxidant capabilities.

Tissue differences for potential peroxidation were apparent. In the present study, fatty acid analysis verified incorporation of dietary fatty acids into colon mucosal microsomes. There was an increased accumulation of linoleic and arachidonic acids in CO-fed animals, an increased omega-3 fatty acid content in MO-fed animals, an increased monounsaturated fatty acid content in BT-fed animals, and a difference in double bond index of the BD and BT groups as compared with the CO and MO groups. In spite of these differences, there were no effects of DMH on colon microsomal lipid peroxidation and the microsomes were resistant to peroxide inducement by iron. Other investigators have found a similar resistance to peroxidation in this tissue. In a study by Balasupramanian et al.,[20] colon microsomes from rats on unspecified diets were shown to be resistant to iron-induced peroxidation. In another study, chow-fed animals showed no change in lipid peroxidation in the colon as the result of DMH treatment until after 15 weeks (15 injections), when lipid peroxidation decreased.[12] Reports by Balasupramanian[20] indicate that intestinal mucosal tissue possesses unique antioxidant capabilities and does not respond to traditional inducers of lipid peroxidation. The intestinal tissue is reported to contain an endogenous inhibitor of peroxidation, which has been found to be a mixture of free monounsaturated fatty acids, specifically oleic and palmitoleic acids.[20] Under the conditions of varied fatty acid composition in the present study, antioxidant protection of the colon is still evident. Colon mucosal tissue would then be less susceptible to toxicity from DMH than liver, as measured by inducible lipid peroxidation. Yet, colon mucosa is more susceptible to DMH-induced tumorigenesis.[2] Therefore, the role of lipid peroxidation as a modulator of DMH-induced carcinogenesis in colon is not apparent.

In summary, liver was shown to be susceptible to microsomal peroxidation, dependent on dietary lipid source, age, and DMH treatment. The colon, however, was highly resistant to peroxidative damage. Based on these data, it does not appear possible to relate colon tumor promotion by dietary fat to lipid peroxidation in this tissue.

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