The Influence of Dietary Medium Chain Triglycerides on Rat Mammary Tumor Development[1]

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The N-nitrosomethylurea rat mammary tumor model was used to compare the tumor-promoting effects of a high-fat (HF) diet containing a 3:1 mixture of medium chain triglycerides (MCT) and corn oil with that of a HF and a low-fat (LF) corn oil diet. The serum and tumor lipid content and fatty acid (FA) composition were also determined in the three dietary groups. It was found that the MCT-containing diet failed to promote tumor development compared with the HF corn oil group. Tumor incidence in the HF-MCT group was similar to that of the LF corn oil group (5% fat, w/w), but significantly decreased compared to the HF corn oil group. Total serum cholesterol levels were significantly depressed in the HF corn oil group compared to the HF-MCT and LF corn oil groups. Analysis of serum and tumor FA profiles indicated that the HF corn oil group exhibited approximately twice the amount of linoleic acid (LA) as the other two treatment groups. Differences among the three groups in the major FA metabolite of LA, arachidonic acid, were minimal. These results are consistent with the hypothesis that tumor promotion by dietary fat is more a function of the type than the amount of fat ingested. In addition, they indicate that MCT, due at least in part to their unique structural and physiological properties, exert markedly different effects on mammary tumor development than conventional long chain unsaturated fatty acids.
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Numerous epidemiological and experimental studies suggest that dietary fat is an important determinant of breast cancer risk (1,2). Studies in laboratory animals have shown that the influence of dietary fat is exerted primarily on the promotion stage of mammary carcinogenesis (3), a finding that may be reflected in the fact that the association between fat intake and increased risk is most pronounced in women over 40 (2).

Moreover, experimental studies by us (4), Carroll et al. (3,5-7) and others (8-15) have shown that the kind and amount of fat ingested determine the rate of development of chemically induced and transplantable mammary tumors. In these studies, it was shown that a certain proportion of essential polyunsaturated fatty acids (PUFA) appears to be required for optimal tumor-enhancing effects, and that high-fat (HF) diets containing coconut oil, which consists primarily of short and medium chain saturated fatty acids and a small quantity (<2%) of linoleic acid (LA), lack the ability to promote mammary tumor development compared to HF diets rich in PUFA.

As the above evidence suggests, the degree of saturation and possibly the chain length of constituent fatty acids can modify the effect of HF diets. Hence, it appeared appropriate to test the effects of a specifically designed diet containing high levels of medium chain triglycerides (MCT) on the development of chemically induced mammary tumors. (Medium chain fatty acids [MCFA] are obtained by hydrolysis of coconut oil—6:0 [<2%], 8:0 [7%], 10:0 [6%], 12:0 [50%], 14:0 [20%], 16:0 [8%], 18:0 [2%], 18:1 [5%], 18:2 [0.8%]—followed by fractional distillation of the resulting fatty acid mixture to obtain a mixture of 6:0 [1-2%], 8:0 [65-75%], 10:0 [25-35%], 12:0 [1-2%] fatty acids. The MCFA are then esterified to glycerol in the presence of a Zn catalyst to generate the triglyceride [17].)

In the present study, the N-nitrosomethylurea (NMU)-induced rat mammary tumor model was used to compare the tumor-promoting effects of diets containing low and high levels of corn oil with a HF diet containing a 3:1 mixture of MCT and corn oil. Since dietary MCT have been reported to alter circulating serum lipid levels (16,17) and to modify the metabolism of linoleic acid (18,19), serum total cholesterol and triglycerides and serum and tumor fatty acid profiles were also assessed.

MATERIALS AND METHODS

Tumor induction. Ninety inbred virgin female F344 rats, aged 28 days (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were maintained on the standard NIH-07 diet (Zeigler Bros., Gardners, Pennsylvania) (20) until 50 days of age. All animals were then randomized into three groups of 30 animals each by recognized procedures (21) to equalize initial mean weights. On day 50 of age, all animals received a single dose of NMU (50 mg/kg body wt) by tail vein injection. The NMU (Ash Stevens Inc., Detroit, Michigan) was dissolved in a few drops of 3% acetic acid, and NMU was brought up to volume in distilled H_2O, yielding a stock solution of 10 mg/ml administered within 2 hr of formulation (22). Two days after carcinogen administration, animals were transferred to experimental diets and remained on them for the duration of the experiment.

At weekly intervals beginning four weeks after NMU injection, each rat was weighed, and the position and date of appearance of palpable tumors were recorded.

HF and low-fat (LF) diets. The adjusted HF diet used in these experiments is based on the recommendations of the Committee on Laboratory Animal Diets of the National Academy of Sciences (23-25), with slight modifications.

It has been found in our laboratory and those of others that rats adjust food intake so that similar energy intake is maintained, despite the fact that diets may differ substantially in energy density. Hence, animals will eat quantitatively less of a HF than LF diet. Consequently, unless the proportions of the other components in the diet are adjusted, animals fed a HF diet will take in substantially less protein, fiber, vitamins, minerals, etc., than

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those fed a LF diet. The adjustment recommended by the Committee on Laboratory Animal Diets was incorporated into our experimental protocol. The adjusted diet formulation ensured that animals fed a HF diet took in the same amount of vitamins, minerals and fiber as in the LF diet and that adequate antioxidant activity was present in the form of vitamin E, Se (as a cofactor of glutathione peroxidase) and vitamin A in each dietary group. The increase in fat in the HF diet was compensated for by a decrease in the amount of starch-dextrose (26) (Table 1). The HF and LF diets consisted of 23% and 5.0% corn oil. These percentages were designed to mimic the American (high risk) diet (40–45% of total calories as fat) and the Japanese (low risk) diet (16% of total calories as fat during the years 1957–1959) (2).

The LF diet was designed to provide ca. 5–6 cal/day of fat, based on an estimated consumption of 45–50 cal/day, (27) or ca. 12% of total. The HF diet, on the other hand, provided ca. 20–21 cal/day as fat, or 45% of total calories. All dietary ingredients were obtained from Dyets Inc. (Bethlehem, Pennsylvania) and were mixed in-house in our diet kitchen. Diets were formulated in 4-kg lots and stored in plastic bags at 4 C in the dark until used.

Animals were housed three to a cage in plastic cages covered with filter tops in a room controlled for temperature (24 C ± 2 C), light (12-hr cycle) and humidity (50%) and were administered diets (in powdered form) and tap water ad libitum.

Histopathology. Approximately 22 wk after NMU administration, all rats were killed by decapitation, and serum was collected following centrifugation of whole blood. Serum was stored at −20 C until assayed. Palpable tumors were excised and cut into two pieces—one of which was stored in liquid N_2 for fatty acid analysis and the other fixed in buffered formalin—blocked in paraffin and then sectioned and stained with hemotoxylin and eosin. Histological diagnosis of mammary tumors was based on the criteria outlined by Young and Hallowes (28).

Statistical evaluation of tumor data. Differences in tumor-free survival time among the three treatment groups were analyzed by the Kaplan-Meier Product Limit Method using a Fortran program provided by G. G. Gart and colleagues (29). Differences in tumor incidence were assessed by Fisher's Exact test (one-tail) and in tumor multiplicity by analysis of variance (ANOVA) after log transformation of the tumor count data. Overall, weight gains among the three treatment groups were compared by a two factor (diet and time) ANOVA with repeated measures (30).

Biochemistry. Nonfasting serum total cholesterol and triglycerides were determined by the use of a Gilford 3500 computer-directed auto analyzer by standard procedures (31,32).

Serum fatty acid analysis was carried out as described previously (4,33). Essentially, pooled serum samples (five/group) were extracted by a modified Radin (34) technique using hexane-isopropanol as the extraction solvent. The extracted lipid was then transmethylated using BF_3 (35), and the methyl esters of the fatty acids were separated by GLC. For tumor lipid analysis, frozen tumor tissue was pulverized under liquid N_2 in a mortar and pestle and then extracted in a similar manner as serum. The total lipid extract was then separated into neutral and phospholipid fractions using a silica gel column (4,36). The column was eluted first with chloroform (neutral lipid) and then a chloroform/methanol/water (65:26:4, v/v/v) mixture (phospholipid). The efficiency of extraction and separation was 94% for neutral lipids and 97% for phospholipids.

RESULTS

Tumor incidence, latency and multiplicity. Animals fed the MCT-containing diet exhibited a significantly lower total mammary tumor incidence when compared to animals fed a HF:corn oil diet (60% vs 87%, $p < 0.03$) (Table 2). However, when palpable adenocarcinomas alone were counted, the difference in incidence failed to attain statistical significance (57% vs 77%, $p < 0.08$). Likewise, when HF corn oil and LF corn oil–fed animals were compared in terms of total palpable mammary tumors, statistical significance was barely missed (66% vs 87%, $p < 0.06$); however, when only palpable adenocarcinomas

TABLE 1

Composition of Defined, Semipurified Diets (AIM-76A)

	Low fat diet	Adjusted high fat diets	
Ingredient	Corn oil[a] (g)	Corn oil[a] (g)	MCT[a] (g)
Casein	20.0	23.5	23.5
Cornstarch	52	32.9	32.9
Dextrose	13	8.30	8.30
Fat			
Corn oil	5	23.52	5.88
Medium chain triglyceride			17.64
DL-Methionine	0.3	0.35	0.35
Choline bitartrate	0.2	0.24	0.24
Alphacel	5	5.9	5.9
(AIN-76) Vitamin mix	1.0	1.18	1.18
(AIN-76) Mineral mix	3.5	4.11	4.11
Total	100.0	100.00	100.00
Energy value (cal/g)	3.89	4.73	4.73

[a]Five percent corn oil is added to assure adequate amounts of essential fatty acids.

were counted, differences in tumor incidence did not reach the level of statistical significance (60% vs 77%, $p > 0.10$).

Analysis of time-to-first-tumor curves (Fig. 1) indicated clearly that mammary tumors appeared more rapidly in the HF corn oil group than in the HF-MCT and LF-corn oil groups. The stepwise survival curves exhibited by the latter two groups were indistinguishable, whereas there was a significant delay in tumor appearance in these groups when compared to the HF-corn oil curve. The median times to first tumor in the HF-corn oil, HF-MCT and LF-corn oil groups were 90, 120 and 120, respectively (Table 3). Tumor multiplicity was similar in each group (Table 4), and no difference in tumor size was seen in the different treatment groups (data not shown).

Biochemistry. With regard to serum lipid concentrations, mean nonfasting cholesterol levels were significantly lower in animals fed 23% corn oil compared to those fed either 5% corn oil or the corn oil–MCT diet. No significant differences were found in serum triglycerides for any of the three treatment groups (Table 5).

TABLE 2

Influence of Medium Chain Triglycerides on the Incidence of NMU-Induced Mammary Tumors[a]

Dietary fat	% Fat	N[b]	Adenocarcinoma	Fibroadenoma	Total tumors
Corn	23	30	77[c] (23/30)[d]	10[c] (3/30)[d]	87[c] (26/30)[d]
Corn	5	30	60 (18/30)	6 (2/30)	66 (20/30)
Corn	6	30	57 (17/30)	3 (1/30)	60 (18/30)
+ Medium chain triglyceride	18				

All adenocarcinoma comparisons NS. Total tumors: 23% corn vs 5% corn = .062; 23% corn vs corn + MCT = 0.03; 5% corn vs corn + MCT = NS.

[a]Palpable tumors only.

[b]No. animals at risk.

[c]Tumor incidence (%).

[d]Number of tumor-bearing animals/number of animals at risk.

TABLE 3

Influence of Medium Chain Triglycerides on the Latent Period of NMU-Induced Mammary Tumors

Dietary fat	% Fat	N[a]	Mean latent period (days postinduction)	Median latent period (days)
Corn	23	30	86 ± 23[b]	90
Corn	5	30	117 ± 36	120
Corn	6	30	122 ± 40	120
+ Medium chain triglyceride	18			

[a]Number of animals at risk.

[b]Mean days to first tumor/±SD.

TABLE 4

Influence of Medium Chain Triglycerides on the Latent Period of NMU-Induced Mammary Tumors

Dietary fat	% Fat	No. adenocarcinoma/total animals with 1 or more adenocarcinomas
Corn	23	0.48 ± 48[a,c] (1.61)[c]
Corn	5	0.43 ± .56 (1.53)
Corn	6	0.58 ± 61 (1.78)
+ Medium chain triglyceride	18	

[a]Least square mean ($\log_e$)±SD.

[b]All pairwise comparisons NS (by one-way analysis of variance).

[c]Anti-$\log_e$.

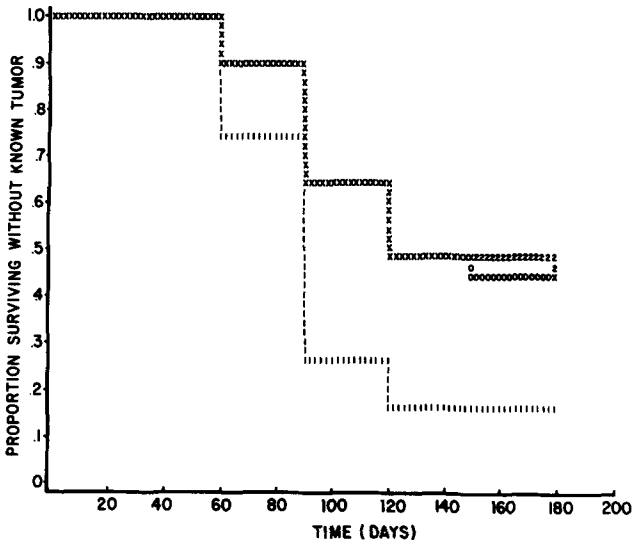

FIG. 1. Kaplan-Meier life tables curves for cumulative mammary tumor incidence. 0, 18% MCT + 6% corn oil; 1, 23% corn oil; 2, 5% corn oil; X, overlapping lines. Tests for overall trend: see ref. 9. Cox's test for adjusted trends, $p < 0.0035$. Pairwise comparisons: Cox's Test (conservative). 23% corn vs 5% corn, $p < 0.0099$; 23% corn vs MCT, $p < 0.0086$); 5% corn vs MCT, $p < 0.9$.

The fatty acid profiles of serum lipids differed primarily in the quantity of linoleic acid (18:2) present. Animals fed 23% corn oil diets exhibited twice the amount of LA as those fed 5% corn oil or MCT-containing diets. Differences in 14:0, 16:0, 16:1, 18:3 and 20:4 fatty acids were also noted (Table 6), though these were of a lower degree of magnitude.

Comparison of the fatty acid profiles in tumor neutral lipids (Table 7) indicated that the spectrum of fatty acids closely reflected that of the diet, particularly with regard to the essential fatty acid linoleic acid (18:2n-6); 10:0 and 12:0 fatty acids were detected only in the MCT group and at very low levels.

Comparison of phospholipid fatty acid profiles (Table 8) in NMU-induced tumors revealed a marked difference compared to neutral lipid profiles. In general, AA levels were higher than LA levels in the phospholipid fraction. When the phospholipid profiles of the three treatment groups were compared, it could be seen that LA still exhibited higher levels compared to the LF-corn oil and the HF-MCT groups. No differences were seen in AA levels, which tended to be highly variable both within and between groups.

Animal weight gains were similar in each group (Table 9), indicating that differences in type or quantity of

TABLE 5

Influence of Various Dietary Fats on Serum Lipid Concentrations

Dietary fat	% Fat	N[a]	Cholesterol	Triglycerides
Corn	23	30	80 ± 16[b,e] (81)[c] (53-137)[d]	117 ± 43[f] (116) (38-199)
Corn	5	30	108 ± 15 (107) (81-146)	112 ± 46 (98) (63-303)
Corn + Medium chain triglyceride	6 18	30	107 ± 15 (109) (66-131)	142 ± 83 (117) (65-420)

[a]Number of animals at risk.
[b]Arithmetic mean ± SD (mg/100 ml).
[c]Median.
[d]Range.
[e]Corn (23) vs MCT $P < .0001$. Corn (23) vs corn (5) $P < .0001$.
[f]All pairwise comparisons NS (by one-way analysis of variance).

TABLE 6

Comparison of Serum Lipid Fatty Acid Profiles in Animals Fed Diets Varying in Type and Amount of Fat

	Fatty acid percentages									
Fat (%)	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Corn (23)	—[a]	—	—	18.4[b] (±0.9)	—	19.1 (±1.5)	9.8 (±1.2)	28.5 (±1.7)	—	23.5 (±2.2)
Corn (5)	—	—	0.5 (±0.1)	22.9 (±0.7)	1.2 (±0.2)	17.6 (±3.9)	15.1 (±1.8)	13.2 (±1.3)	0.5 (±0.3)	29.1 (±4.4)
Corn (6) + MCT (18)	—	—	0.7 (±0.2)	24.5 (±5.0)	1.1 (±0.3)	19.4 (±1.4)	11.4 (±2.6)	13.3 (±2.7)	—	28.2 (±0.9)

[a]—, Not detectable.
[b]Percentage of total fatty acids; mean ± SD (five pooled samples/group).

TABLE 7

Tumor Neutral Lipid Fatty Acid Profile[a]

	Fatty acid percentages									
Fat (%)	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Corn (23) (n = 9)	—	—	1.4[c] (±0.79)	19 (±2.2)	1 (±0.26)	4.6 (±1.2)	28 (±1.5)	43 (±6.5)	1.0 (±0.9)	2.3 (±2.2)
Corn (5) (n = 8)			2 (±0.58)	26 (±1.9)	4.8 (±1.9)	3.7 (±1.4)	33 (±3.9)	28 (±4.7)		1.5 (±2.2)
Corn (6) + MCT (18) (n = 11)	1.0 (±0.7)	0.4 (±0.1)	2.5 (±.79)	29 (±2.8)	3.0 (±0.8)	7 (±2.8)	34 (±2.9)	19 (±6.3)	0.8 (±.67)	5.3 (±4.8)

[a]Percentage of total fatty acids.
[b]—, Not detectable.
[c]Mean ± SD.

TABLE 8

Tumor Phospholipid Fatty Acid Profile

	Fatty acid percentages									
Fat (%)	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4
Corn (23) (n = 8)	—[a]		1.5 (±2.2)	34[b] (±2.2)	1.0 (±0.1)	18 (±6.7)	19 (±4.8)	11[c] (±7.4)	—	17 (±9)
Corn (5) (n = 10)			1.0 (±0.6)	33 (±2.8)	1.3 (±0.6)	16 (±1.8)	21 (±3.9)	4 (±1.7)	—	24 (±6.5)
Corn (6) + MCT (18 (n = 10)		—	1.2 (±0.6)	33 (±8.2)	2 (±0.9)	16 (±5.7)	26 (±5.6)	5 (±2.4)	1 (±0.6)	17 (±8.6)

[a]—, Not detectable.
[b]Percentage of total fatty acids; mean ± SD.
[c]LA content: corn (23) vs corn (5), $p < 0.02$; corn (23) vs MCT, $p < 0.034$; corn (5) vs MCT, NS (all by two-tailed t-test).

TABLE 9

Cumulative Mean Animal Weights (g)

Weight (weeks postinduction)	Experimental group		
	Corn (23%)	Corn (18%) + MCT (6%)	Corn (5%)
0	100 ± 4	100 ± 4	101 ± 3
4	137 ± 6[a]	137 ± 7	135 ± 6
8	163 ± 9	161 ± 9	159 ± 7
12	169 ± 10	166 ± 10	165 ± 6
16	175 ± 10	176 ± 10	171 ± 8
20	176 ± 10	178 ± 12	175 ± 9
22	178 ± 11	178 ± 14	176 ± 10

Not significant by analysis of variance.
[a]Arithmetic mean ± SD; all comparisons of weight curves.

dietary fat did not alter food consumption patterns. Direct measurement of food consumption confirmed this finding: LF-fed animals consumed 11-12 g/day while HF-fed animals consumed 8-10 g/day.

DISCUSSION

This study indicates that a HF diet containing high levels of MCFA does not enhance the development of mammary tumors, in contrast to a HF diet containing the more common long chain fatty acids. Possible mechanisms underlying the observed effects of the MCT-containing diet may be either direct or indirect. A direct mechanism can be envisaged based on the unique physicochemical and biological properties of MCT. Although lipid in nature, MCT are absorbed and transported by the body in a manner more characteristic of carbohydrates than lipids. In contrast to long chain fatty acids, MCT do not enter lymph or chylomicrons, are not incorporated into membranes and are rapidly oxidized by the mitochondria via a carnitine-independent, rather than a carnitine-dependent, mechanism (16,17,37). The presence of only small amounts of MCFA in serum or tumor lipids of MCT-fed animals indicates that MCFA are rapidly absorbed and oxidized rather than stored in tissues. Hence, because of the unique physiological properties of MCT, the HF-MCT diet may exert biological effects more like those of a LF than a HF diet.

MCT may also act via indirect mechanisms involving actions at hormone receptors and/or essential fatty acid metabolism. With regard to the former, it has been shown by Knazek et al. (38) and Cave and Erickson-Lucas (39) that feeding MCT to rats lowered the number of prolactin receptors in both hepatic tissue and mammary tumors. Since prolactin is a recognized promoting substance in mammary tumorigenesis (40,41), its tumor growth-promoting effects could be attenuated by limiting, via dietary means, the number of receptors in the target organ available for activation by circulating prolactin. With regard to the latter, there have been several reports that MCT influence essential fatty acid metabolism (18,19). They have been shown, for example, to exert a sparing effect on the LA requirement for relief of the symptoms of essential fatty acid deficiency. These findings are of particular interest since essential fatty acids and their metabolism to prostaglandins appear to play a role in both the HF effect and the essential fatty acid deficiency syndrome (10,42,43-45).

Differences in serum and tumor fatty acid profiles in the three treatment groups were seen mainly in the LA content, which was significantly elevated in the HF-corn oil group. Of all the major fatty acids, only LA was elevated in the 23% corn oil animals. This fact is probably a reflection of its status as an essential fatty acid—that is, it cannot be synthesized de novo or from dietary precursors by the body, and therefore must be performed in the diet (43). Since Δ^6-desaturase (the rate-limiting enzyme in the pathway from LA to arachidonic acid [AA] and ultimately the whole range of prostaglandins), is subject to inhibition by long chain fatty acids (46), consumption of a HF-corn oil diet may suppress the activities of the fatty acid desaturases and/or chain-elongating enzymes and thereby slow the conversion of LA to its various metabolites. However, in the absence of any direct data on the fatty acid desaturases, one can only speculate on the metabolic basis for the observed differences in fatty acid patterns reported in the present study.

The fact that AA levels in tumor phospholipids (the primary precursor for intracellular prostaglandins) were similar in all three groups, despite the observed differences in tumor yield, casts doubt on the possibility that mammary tumor promotion may be regulated by the amount of AA available in membrane phospholipids for conversion to prostaglandins (43,47,48), and also on the possibility that dietary MCT modulate the metabolism of LA to AA in mammary tumors.

The intake of MCT has been associated with reductions in tissue and serum cholesterol (16). In this study, animals receiving the MCT-containing HF diet exhibited significantly higher, rather than lower, serum cholesterol compared to animals receiving 23% corn oil. The reason for this is uncertain. It may be because MCT limit cholesterol deposition in tissues (4) and the observed high serum cholesterol levels may therefore be due to a reapportionment of cholesterol from the tissue to the serum compartments. The suppression of serum cholesterol levels by diets high in polyunsaturated fat, such as corn oil, is a well-established phenomenon, although the mechanism by which this occurs is uncertain (49,50).

In conclusion, this study emphasizes the importance of viewing dietary lipids not only in terms of their physicochemical characteristics, i.e., chain length, degree of saturation and levels of isomerization, but also in terms of their specific physiological roles in body metabolism. In this regard, MCT, which are unique among triglycerides in their mode of absorption and transport, should provide a valuable tool for further exploration of the role of dietary fat in breast cancer development.

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