Interrelations of Linoleic Acid with Medium-Chain and Long-Chain Saturated Triglycerides[1]

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MEDIUM-CHAIN (C_6–C_{12}) saturated triglycerides (henceforth MCT) are present in most naturally occurring fats and thus have found the attention of biochemists and nutritionists for the last 50 years. Some of the findings have been that they play a part in germinating cells (10), are more readily absorbed than long-chain saturated triglycerides (LCT) (21, 6), are absorbed by blood vessels rather than lymph vessels as is LCT, and are metabolized by *omega* oxidation (2). There seems to be little tendency for 8 and 10 carbon saturated acids to be laid down in the depot fat (8, 15); the opposite is true of C_{16} and C_{18} acids. In this respect and other respects C_{12} and C_{14} acids may form the border between the two groups (7).

In our previous work with LCT and MCT various differences in their biological effects had also become apparent (14, 15); some of these seemed to have been related to the linoleic acid content of the diet. It was therefore decided to investigate in rats the possible relationship between linoleic acid and MCT and LCT in studies of growth, reproduction, and serum and liver cholesterol levels. The results are reported in this paper.

Experimental Procedures and Data

General Procedures. The current studies consisted of a series of experiments. In the first, low-fat and 20% MCT diets supplemented with 0, 0.1, and 2% linoleic acid (LA) were fed to weanling male rats for 126 days, and the animals were observed for growth, liver and serum cholesterol levels, and liver lipides. In a second experiment rats were fed low-fat and 20% LCT diets containing no added LA; this was followed by another series in which rats were fed low-fat and 20% LCT diets supplemented with either 0 or 2% LA. All of these groups were observed over a period of 7–8 weeks for growth and, at the termination of the experiment, for serum and liver cholesterol levels and liver lipides.

Mating experiments were carried out on male and female rats fed low-fat, 20% MCT, and 20% LCT diets containing various levels of LA.

Materials and Methods. The medium-chain and long-chain saturated triglycerides were derived from coconut oil or other kernel oils by fractionation of the split fatty acids and reconstitution of the desired fractions into triglycerides. MCT represented about 15% of the original coconut oil and was a clear, thin, odorless liquid with a melting point below 0°C. and an iodine number less than 1. Ultraviolet analysis showed that 0.1% linoleic acid was present although in one lot, 0.6% was found. The oleic acid content was in the neighborhood of 0.1%. The saturated fatty acids present ranged from C_6–C_{12} and represented more than 99% of the fatty acids present in the product.

The LCT fraction represented about 60% of the original coconut oil and was a solid fat having a melting point of about 40°C. and an iodine number of 3–5. The saturated fatty acids ranged from C_{14}–C_{18} and represented more than 95% of the fatty acids in the product. The oleic acid content was 1–2%, and the linoleic acid content 0.2–0.4%.

MCT and LCT were incorporated at a level of 20% in the purified diet given in Table I. The casein was alcohol-extracted. The diet was supplemented with all accessory factors except LA unless otherwise noted. When it was desired, fat was replaced by carbohydrate. Larger amounts of vitamins A and E were given in the low-fat diet to ensure adequate absorption.

TABLE I

Composition of Purified Rat Diet

Dextrose	44%	Fat	20 %
Vitamin-free casein	30%	Salts (USP XIII)	3.5%
Cellulose	2%	$CaCO_3$	0.5%
Water-soluble vitamins [a]		Fat-soluble [b]	
	Mg./kilo.		*Mg./kilo.*
Choline dihydrogen citrate	1000	Alpha-tocopherol acetate	50
Inositol	1000	Free alpha-tocopherol	10
p-Amino benzoic acid	300	Vitamin D_2	.5
Nicotinic acid	100	Beta-carotene	5
Thiamine	2		
Riboflavin	4		
Pyridoxine	4		
Calcium pantothenate	10		
Folic acid	2.5		
Biotin	.025		
B_{12} (.1% trituration)	5		
Ascorbic acid	25		

[a] We are indebted to Leo Pirk, Hoffmann-LaRoche Inc., Nutley, N. J., for most of the vitamins and to M. L. Tainter, Sterling-Winthrop Research Institute, Rensselaer, N. Y., for the vitamin D_2.

[b] Contained in 1 cc. of an MCT suspension.

The experiments were carried out on albino rats of the Sherman strain. For the comparison of the effects of low-fat, MCT, and LCT diets the offspring of females maintained on MCT without added LA were used. They were placed on the experimental diets at four to five weeks when they weighed 40–50 g. This was done in order to start the experiment with essential fatty acid-depleted animals. The male rats used for the long-range survival studies were born on commercial pellets and placed on the experimental diets when they were weaned.

Serum cholesterol determinations were carried out according to Schoenheimer and Sperry (23) and liver and heart analyses, according to Sperry and Webb (25). Three or four sera or livers or six to eight hearts were pooled for analysis.

Data and Discussion. In Table II are given the growth data for male rats on MCT, LCT, and low-fat

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TABLE II

Average Body Weights, in Grams, of Male Rats Fed Purified Diets Containing no Added Fat, 20% MCT, or 20% LCT and Various Supplements of Linoleic Acid (LA)

	Low-fat +			20% MCT +			20% LCT +	
	0% LA	0.1% LA	2% LA	0% LA[c]	0.1% LA	2% LA	0% LA	2% LA
Series I (154 days)[a] 8 rats on each diet	223 ± 15.2[b]	286 ± 11.7	317 ± 10.3	301 ± 17.6	324 ± 7.5	336 ± 9.4	......	......
Series II (79 days) 4 rats on each diet	191 ± 14.8	......	......	......	......	......	166 ± 14.5	......
Series III (84 days) 4 rats on each diet	200 ± 8.1	......	232 ± 10.0	......	......	......	163 ± 10.0	270 ± 8.7

[a] Age at termination of experiment. [b] Standard error. [c] Inclusion of 20% MCT or LCT increased the linoleic acid content of the diets by approximately 0.02 and 0.05% respectively.

diets with and without LA supplements. One must keep in mind when comparing the effect of MCT diets with those of the low-fat diets that it was necessary to compensate for the differences in linoleate intake of the animals on the different diets. Inasmuch as the daily food intake of the adult males on the diet with 20% MCT was about 12 g. (13), they consumed about 2.5 mg. of linoleic acid and may have occasionally had as much as 10 mg. from this source. The alcohol-extracted casein contained about 0.5% fat. If one assumes that this fat contained about 10% linoleic acid, the 30% casein in the diet would have afforded 3 mg. per day. Therefore these rats consumed about 5–10 mg. of linoleic acid daily. The corresponding animals on the low-fat diet ate about 17 g. of food a day, from which they may have received 3 mg. of linoleate. Therefore some groups on the low-fat diet were given 0.05 and 0.1% LA, which allowed an additional daily intake of 7–15 mg.

In Table II it can be seen that, among the animals in Series I, those fed the 20% MCT diet without added linoleic acid grew significantly better than those on the unsupplemented low-fat diet (P less than .01) and at least as well as those on the low-fat diet supplemented with 0.1% LA. In Series II the animals fed 20% LCT unsupplemented with linoleic acid grew worse than the corresponding animals on the low-fat diet; this was also true for Series III (P less than .02 for the averages of the two series). This was in spite of the fact that the LCT contained some linoleic acid. On the other hand, in Series III, the animals fed LCT + 2% LA grew better than the corresponding group fed the low-fat diet. This permits the conclusion that the low weight of the animals on the unsupplemented LCT diet was an expression of enhanced linoleic acid requirement (5). With sufficient linoleic acid they were heavier, as has been observed for animals on any high-fat diet (22). MCT did not increase the linoleic acid requirement or aggravate the linoleic acid deficiency symptoms.

It had to be considered that differences in intestinal absorption may have accounted for these and subsequent results. In earlier work intestinal absorption of MCT was determined according to a modification of the method of Hoagland and Snider (11, 12). The fecal fat determination was done on an aliquot of the finely powdered, dried feces. The samples were treated with 5% trichloracetic acid; the latter was washed out, and the residues were saponified with methyl alcoholic NaOH. After acidification the extracts were extracted with petroleum ether, dried, and weighed. MCT was 98% absorbed (14). In the LCT absorption studies the food intake of eight rats fed the 20% LCT diet was measured, and their feces were collected for the same period. The eight rats consumed 45.3 g. of diet per day or 9.06 g. LCT. The dried feces weighed 2.00 g. and contained 0.16 g. of fat. Therefore, out of 9,060 mg. of LCT eaten, a maximum of 160 mg., or 2%, was excreted (98% absorption). Correction for excretion of endogenous fat was omitted; it would have resulted in a higher figure for the absorption. Inasmuch as the LCT contained 1–2% oleic acid in addition to at least 95% saturated C_{16} and C_{18} acids, the high rate of absorption of LCT may have been partly caused by the oleate.

TABLE III

Survival Rate of Male Rats Fed 20% Lard or 20% MCT with Various Linoleic Acid (LA) Supplements

Age (days)	300	550	700
20% lard	16/22	13/19[a]	10/19
20% MCT + 0% LA[b]	3/4	3/4	......
20% MCT + 0.1% LA	28/30	15/18	14/18
20% MCT + 2% LA	7/7	5/7	......

[a] A smaller denominator means that animals were killed for some other purpose, such as organ analyses.
[b] Regardless of supplementation, inclusion of 20% MCT gave a linoleic acid level of 0.02% in the diet.

Table III gives data as to the longevity of male animals on MCT with and without LA supplementation and of their controls on lard. It is evident that the survival rate of all MCT groups was at least as good as that of the rats fed lard. In fact, in the experiment in which observations were carried out for two years, only one-half of the lard animals but three-fourths of those on MCT survived.

TABLE IV

Influence on Reproduction of Linoleic Acid (LA) Added to 20% MCT, 20%LCT, and Low-Fat Diets

	No. of litters	No. of litters with dead young	Av. no. of live young in litters	Av. no. of young after 4 weeks	Av. wt. of young at 4 weeks
					g.
Low-fat + 0% LA	6/8[a]	4/6[b]	3.5	1.5	35
Low-fat + 0.05% LA	7/8	2/7	6.8	4.8	35
Low-fat + 0.1% LA	7/8	2/7	7.9	4.7	26
Low-fat + 2% LA	3/4	0/4	6.4	5.3	45
20% MCT + 0% LA[c]	47/65	9/47	6.8	3.0	45
20% MCT + 0.1% LA	63/92	12/63	7.3	5.3	48
20% MCT + 2% LA	11/15	1/11	8.0	6.0	64
20% LCT + 0% LA	7/8	2/7	6.0	0.4	31
20% LCT + 2% LA	5/6	0/5	8.2	5.8	60

[a] Six out of 8 mated females had litters.
[b] Four out of the 6 litters contained dead young.
[c] Regardless of supplementation, inclusion of 20% MCT or LCT increased the linoleic acid content of the diets by 0.02 or 0.05%, respectively.

In Table IV are summarized the results of mating experiments with females which had been kept on the various diets for one to three months prior to the mating. With the low-fat diets 23 out of 28 animals became pregnant; there were more litters with dead young in the groups with no or low LA supplements than in the group given 2% LA. LA supplementation reduced the number of litters with dead young from 4 out of 6 to 0 out of 3 and increased the average number of live young per litter from 3.5 to 6.4 and the number of surviving young after 4 weeks from 1.5 to 5.3. However the weaning weights increased only from 35 to 45 g. These findings are in agreement with other studies of the influence of fat and linoleate on fertility, reproduction, and lactation in various species. It has been observed that lactation is improved by dietary fats containing linoleate and that a low-fat diet restricts milk production severely (20, 19). On the other hand, conception is hardly influenced by a low-fat diet (4, 9). Most of the young die soon after birth, and the few survivors are undersized.

In the animals fed MCT and no LA, which are best compared with the low-fat group given 0.05% LA, 47 out of 65 females become pregnant, which is about the same percentage as in the comparable low-fat group. The average number of live young and the survivors after 4 weeks were 6.8 and 3.0, respectively—not very different from those of the low-fat group. The weaning weight of the animals on MCT was 45 g., which is significantly higher than that of the low-fat group. With MCT plus 2% LA the average number of live young was 8.0, and 6.0 survived after 4 weeks. Their weaning weight of 64 g. was significantly higher than that of the group on the comparable low-fat diet.

With unsupplemented LCT 7 out of 8 females had litters; after 4 weeks an average of 0.4 animals survived, which is significantly less than in the low-fat and MCT groups. The weaning weight of the survivors was 31 g., which was significantly less than that of the comparable MCT animals. With 2% LA results were the same as with MCT plus 2% LA. This is in agreement with observations that coconut oil and hydrogenated corn oil intensify the effects of essential fatty acid deficiency (3, 18).

Low-fat and MCT diets differed only in that the weaning weights of the young were better with MCT. This was not merely a general fat effect because, with the unsupplemented diets, LCT did not promote better growth. Yet, with the diets supplemented with 2% LA, MCT and LCT were equally better than low fat.

Data as to serum and liver cholesterol and total liver lipides are given in Table V. In view of the fact that the unsupplemented MCT and LCT diets contained traces of linoleic acid, the most comparable low-fat diet is that containing 0.1% LA. The total liver lipide of the group fed this diet was significantly lower (P less than .01) than those of the groups fed the unsupplemented MCT and LCT diets. With 2% LA the difference persisted; moreover the total liver lipide level of the group on LCT was significantly higher (P less than .01) than that of the MCT group.

The liver cholesterol levels of the groups fed the low-fat diets declined with increasing LA supplements; the differences between the level of the unsupplemented group and of those given supplements were both statistically significant. This is in agreement with previous observations (24, 1). The same linoleic acid effect was observed with LCT; addition of 2% LA led to a significant decline in liver cholesterol (P less than .01). Unsupplemented LCT led to the highest liver cholesterol value. It was significantly higher (P less than .01) than the levels with unsupplemented MCT and low-fat plus 0.1%. The liver cholesterol levels of the groups fed MCT did not differ significantly with LA supplementation and approximated the levels found in the groups fed the other diets plus 2% LA.

TABLE V

Total Liver Lipide and Liver Serum Cholesterol Levels of Male Rats Fed Purified Diets Containing no Added Fat, 20% MCT, or 20% LCT and Various Supplements of Linoleic Acid (LA)

	No. of determinations[a]	Serum cholesterol	Liver lipide		
			Total lipide	Cholesterol	Cholesterol
		mg. %	*% dry wt.*	*mg.% dry wt.*	*% tot. lipide*
Low-fat+0% LA	9	55±2[b]	24.7±0.5	1019±39	4.13±0.16
Low-fat+0.1% LA	3	56±1	23.9±0.5	887±35	3.72±0.21
Low-fat+2% LA	5	87±3	24.6±0.3	810±36	3.30±0.15
20% MCT+0% LA[c]	5	66±3	26.5±0.3	844±38	3.18±0.04
20% MCT+0.1% LA	3	79±3	24.9±0.7	769±24	3.09±0.07
20% MCT+2% LA	3	85±3	26.0±0.3	798±9	3.07±0.06
20% LCT+0% LA	4	74±8	25.6±0.4	1040±14	4.04±0.02
20% LCT+2%LA	2	88±4	28.5±0.3	865±38	3.04±0.10

[a] For each determination, four livers or sera were pooled.
[b] Standard error.
[c] Regardless of supplementation, inclusion of 20% MCT or LCT increased the linoleic acid content of the diets by 0.02 or 0.05%, respectively.

Serum cholesterol levels with MCT and low-fat diets were significantly increased by addition of 2% LA; this is in agreement with reports as to the influence of linoleic acid on serum cholesterol levels (16). However addition of 2% LA to the LCT diet did not lead to a significantly higher level. Serum levels were the same for all groups fed diets plus 2% LA but differed when little or no LA was given. Unsupplemented MCT and LCT led to significantly higher levels than did low-fat plus 0.1% LA. There seems to be a difference in the MCT and LCT levels, but the difference is not statistically significant.

It can be concluded that, in the presence of low levels of linoleic acid, MCT led to significantly lower liver cholesterol levels than LCT and perhaps even to values lower than with low-fat diets, which supports the theory that MCT has a linoleic acid-sparing effect. Whether or not the presence of some oleate in both the MCT and LCT influenced the cholesterol levels is not certain.

The data permit the conclusion that MCT has effects on the animal which are, in some respects, opposite to those of LCT. This antagonism is evident in the relation of LCT and MCT to polyunsaturated acids but has also been observed in other respects. In our own work it was found that MCT promotes growth better than does LCT (15), that MCT protects the animal against the effects of highly rancid fats whereas LCT aggravates the condition (14). LCT increases the tendency of the animal to deposit neutral fat; MCT does the opposite (15, 26). Some of the differences observed by others have been cited in the introduction.

Summary

1. Effects of medium-chain (C_6–C_{12}) saturated triglycerides (MCT) and long-chain (C_{14}–C_{18}) saturated triglycerides (LCT) with and without linoleic acid (LA) supplementation were studied on rats fed purified diets.
2. With 2% linoleic acid rats fed MCT and LCT grew somewhat better than those on a low-fat diet with the same supplement. Without linoleic acid those fed MCT grew better, and those fed LCT grew worse than those on the corresponding low-fat diet. MCT seemed to decrease, and LCT to increase linoleic acid requirements.

3. In survival studies 14 out of 18 rats fed 20% MCT were alive after 2 years; of their controls fed 20% lard, 10 out of 19 survived.
4. Reproduction studies in females gave equally poor results on unsupplemented low-fat, MCT, and LCT diets regarding implantation, birth weight, and survival rate. The weaning weights of the young on MCT were however the highest. With 2% LA weaning weights were equally high with LCT and MCT but lower with low-fat diet.
5. In animals fed low-fat diets not supplemented with LA, low serum cholesterol was associated with high liver cholesterol. With MCT, serum values were higher and liver values were significantly lower. With unsupplemented LCT, serum and liver values were high. When the three diets were supplemented with 2% LA, there were no longer any differences in the serum levels and in the liver levels. Whether or not the presence of some oleate in the MCT and LCT influenced the cholesterol results is not certain.
6. The differences in the effects of MCT and LCT are discussed.

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Glyceride Structure of Vegetable Oils by Countercurrent Distribution. IV. Cocoa Butter[1,2]

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COCOA BUTTER is a fat of unusual physical properties. It melts completely at 33°C. or slightly below body temperature; it is a hard brittle solid at normal room temperatures. Cocoa butter is used in confectionery products largely because its physical properties contribute to glossy coatings, absence of stickiness, and favorable volume changes in the molding operation. Because of the demand for the properties cocoa butter imparts, large quantities of the bean are imported even when domestic fats are in plentiful supply.

The unique melting characteristics of cocoa butter are a consequence of the arrangement of the fatty acids in its glycerides. This fact is illustrated by comparison of cocoa butter with mutton tallow, which is similar to it in fatty acid composition but different in physical properties and unsuitable for use as a confectionery fat. Recent work has shown that oleic acid is predominately in the 2 position in cocoa butter (3, 11, 12, 15) and that palmito-oleo-stearin is the main glyceride.

The glyceride composition of cocoa butter has been investigated by Hilditch and Stainsby (8) and by Meara (13), using fractional crystallization techniques. Recently countercurrent distribution has been shown to be an effective technique for the fractionation of glyceride oils (5, 16, 17). Experimental results obtained with this technique and described and discussed in this paper, are in general agreement with previous data of Hilditch (8) and of Meara (13).

Experimental

Iodine values were determined by the Wijs procedure (1), which was scaled down to allow for the small samples available. Linoleic acid was determined by the 45-min. alkali isomerization method of Brice *et al.* (2). Oleic acid content was calculated from the iodine value and the linoleic acid content, and saturated acids are reported as the difference between the sum of the unsaturated acids and 100%. Stearic acid was determined by a modification of the Nijkamp chromatographic procedure (14). Palmitic acid was

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[3] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.