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Effect of Dietary Medium Chain Triglyceride on Lipogenic Enzyme Activity in Rat Liver

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Summary This study was conducted to confirm that medium chain triglyceride (MCT) feeding itself would increase hepatic lipogenic enzyme activity without causing a lack of essential fatty acids (EFA) in the liver.

1. Male weaning rats were fed for 11 weeks on diets containing 2% corn oil and 13% various fats: MCT, corn oil, tripalmitin or beef tallow, respectively. MCT feeding was clearly shown to increase the activities of fatty acid synthetase (FAS) and malic enzyme (ME) in the liver.
2. Rats weighing 170 g were pair-fed for 7 days on diets containing 15% MCT, 13% MCT + 2% corn oil, 15% corn oil, and 2% corn oil and no fat (control groups), respectively, under a fixed level of carbohydrate (sucrose). The addition of 2% corn oil to MCT (13%) did not depress these enzyme activities, even though supplementing the fat-free diet with 2% corn oil resulted in a significant decline in the activities.
3. When the rats recieved various amounts of MCT, the extent to which the degree of FAS and ME activities increased by MCT feeding depended on the amount of MCT in the dietary fat mixture with corn oil. Supplement of over 5% corn oil to MCT diets did not inhibit them sufficiently. In the liver lipids of animals fed MCT, there were no appearances of 20:3ω9 (5,8,11-eicosatrienoic acid), but the levels of mono-unsaturated fatty acids were increased by MCT feeding.

The results suggest that MCT ingestion itself enhances lipogenic enzyme activity *via* some metabolic charge.

Key Words dietary MCT, hepatic lipogenic enzyme, fatty acid synthetase, malic enzyme, fatty acid composition, liver lipid, rat

The effect of dietary medium chain triglyceride (MCT) on lipid metabolism has received considerable attention in recent years. We have observed the effects of dietary MCT on hepatic enzymes catalyzing lipogenesis and cholesterogenesis in

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Table 1. Basal diet composition.

Ingredient	Fat-free (g/100 g diet)
Sucrose	57
Casein[1]	20
Cellulose powder[2]	18
Salt mix[3]	4
Vitamin mix[4]	0.5
Choline chloride	0.5

[1] Nutritional Biochemical, Cleveland, Ohio. [2] Toyo Roshi Co., Tokyo. [3] Harper's salt mixture purchased from Oriental Yeast Co., Yokohama. [4] The following vitamins were made up to 50 g by adding glucose: Vitamin A acetate, 434 mg; vitamin D_2, 65 mg; vitamin E acetate, 3.1 g; vitamin K_3, 20 mg; niacin, 7.38 g; inositol, 3.69 g; pyridoxine-HCl, 260 mg; thiamine-HCl, 260 mg; riboflavin, 260 mg; calcium pantothenate, 550 mg; *p*-aminobenzoic acid, 3 g; ascorbic acid, 250 mg; vitamin B_{12}, 0.5 mg; biotin, 0.25 mg; folic acid, 25 mg.

rats (*1*). MCT-fed rats have shown high activities of lipogenic enzymes such as FAS ans ME, and low activity of 3-hydoxy-3-methylglutaryl CoA reductase in livers. Kritchevsky and Tepper (*2*) have reported that the incorporation of acetate-1-^{14}C into fatty acids both *in vivo* and *in vitro*, was enhanced by MCT ingestion. Hepatic fatty acid mono-desaturation was enhanced by feeding with MCT (*3*). These phenomena seem to be very similar to those observed in essential fatty acid (EFA)-deficient rats fed a fat-free diet (*4*, *5*), except that the EFA-deficient rats develop fatty livers (*5*), while MCT feeding does not induce such an anomaly (*6*). The mechanism involved in the increase of hepatic lipogenesis induced by MCT remains to be elucidated.

This led us to question: (a) whether or not MCT feeding would lead to a reduction or a lack of EFA in liver lipids in some mechanism(s), followed by an enhancement of lipogenesis; and (b) if MCT feeding itself would cause increasing lipogenesis *via* some metabolic change(s) in animals, induced by alterations of a number of metabolic parameters such as hormones and others as a consequence of the ingestion of MCT. It is well known that MCT is metabolized in a different way from long chain triglycerides (*7*).

The study to be reported here was undertaken in an effort to further confirm the influence of MCT on liver lipogenic enzymes focusing on the questions mentioned above.

METHODS

Experimental animals. Three experiments were carried out. Male Wistar strain rats (Shizuoka Laboratory Animal Center, Hamamatsu) were used. Six rats

per treatment in all experiments were individually housed in wire cages in a room controlled at 23°C with 55% relative humidity. Animals were fed on a commercial stock diet (Oriental Yeast Co., Tokyo, No. MF) for three days to acclimate them to their surroundings. They were then randomly divided into subgroups and fed the various experimental diets described in the following. Deionized water was provided *ad libitum* throughout the experiments.

Experimental design and diets. The experimental diets were formulated by supplementing various fats to a basal diet (fat-free diet), replacing equivalent amounts (by weight) of cellulose. Therefore, in all of the diets, the levels of carbohydrate (sucrose), protein, minerals and vitamins were similar to those in the basal diet. The basal diet is shown in Table 1.

Experiment 1: *Long-term feeding of dietary MCT.* Animals weighing about 40 g after weaning were fed the diets *ad libitum* for 11 weeks in which four types of dietary fats were added to yield a final concentration of 15% fat. The various fats added to the experimental diets were 15% corn oil (corn oil group), 13% MCT + 2% corn oil (MCT group), 13% tripalmitin + 2% corn oil (tripalmitin group) and 13% beef tallow + 2% corn oil (beef tallow group). As EFA deficiency has been shown to influence fatty acid synthesis (*4,5*), corn oil was included at a 2% level in all diets in order to supply the EFA. MCT (Ono Pharmaceutical Co., Osaka) contained only trioctanoate based on GLC analysis. Tripalmitin was of reagent grade (Wako Pure Chemical Industries, Ltd., Osaka).

The food consumption and body weight were measured at 3- or 4-day intervals.

Experiment 2: *Short-term feeding of dietary MCT.* Animals weighing about 170 g were fasted for 2 days and then refed the diets for 7 days by pair-feeding to obtain a fixed level of carbohydrate (sucrose) intake. The diets in this series of the experiments included 15% corn oil, 15% MCT or 13% MCT + 2% corn oil for the experimental groups. The 2% corn oil or fat-free diet served as controls.

Experiment 3: *A dose-response study.* After 2 days of fasting, animals weighing about 150 g were pair-fed the diets for 10 days. The experimental diets included 0, 5, 10 or 15% MCT, and corn oil to achieve a 15% dietary fat concentration in each diet. The 2% corn oil and fat-free diets were employed as controls. The food intake and body weight of each of the animals in experiments 2 and 3 were measured daily during the experimental periods.

Enzyme and lipid analyses. At the end of each experiment, the animals were sacrificed by decapitation and the livers were quickly removed, rinsed with cold saline, blotted and weighed. All tissue preparation procedures were done at 0–4°C.

One portion of each liver was homogenized into two volumes of buffer containing 0.25 M sucrose, 0.1 M potassium phosphate buffer (pH 7.4), 0.07 M $KHCO_3$, 1 mM EDTA, and 1 mM dithiothreitol. After centrifugation of homogenate at 8,000 × *g* for 20 min, the resulting postmitochondrial supernatant was centrifuged at 105,000 × *g* for 60 min. The clear supernatant was removed, with care being taken not to disturb the pellet or the floating fat layer. The soluble supernatant was used to determine the activity of FAS and ME spectrophotometrically as described by

Table 2. Effect of MCT feeding on lipogenic enzyme activity in rat liver (11 weeks).

Parameters	Diet treatment[1]			
	MCT	Corn oil	Tripalmitin	Beef tallow
Final body weight (g)	$312 \pm 5^{2,ab}$	$343 \pm 20^{5,a}$	286 ± 13^{b}	293 ± 15^{ab}
Weight gain (g)	261 ± 8^{ab}	285 ± 21^{a}	230 ± 12^{b}	273 ± 15^{ab}
Food intake (g)[3]	$1{,}029 \pm 13^{a}$	$1{,}103 \pm 18^{b}$	$1{,}006 \pm 10^{a}$	$1{,}012 \pm 21^{a}$
Carbohydrate intake[3] (g/100 g B.W.)	188 ± 7^{a}	183 ± 13^{a}	201 ± 10^{a}	197 ± 11^{a}
Liver enzyme activity (nmol/min/mg protein)				
FAS[4]	26.0 ± 2.5^{a}	15.8 ± 2.9^{b}	14.0 ± 2.0^{b}	13.6 ± 1.0^{b}
ME[4]	150 ± 10^{a}	83 ± 5^{c}	107 ± 12^{bc}	112 ± 5^{b}

[1] Diets contained 57% sucrose, 20% casein and 15% dietary fat consisting of 2% corn oil and 13% MCT, corn oil, beef tallow or tripalmitin, respectively. [2] Data expressed as mean ± SEM of six rats. [3] Figures for food and carbohydrate (sucrose) intake over 11 weeks. [4] FAS and ME indicate fatty acid synthetase and malic enzyme, respectively. [5] Within a row, values not sharing a common superscript letter are different ($p < 0.05$).

Muto and Gibson (*4*).

Protein was measured by the method of Lowry *et al.* (*8*) using bovine serum albumin as a standard.

A second portion of the whole livers of rats in experiment 3, kept at −20°C until use, was used to analyze the fatty acid composition in the total lipid fraction.

Liver lipid was extracted by the method of Folch *et al.* (*9*). Hydroquinone (10 mg per liver) and arachidic acid (5 mg per g liver, Applied Science Lab., Inc.), as an internal standard for GLC analysis, were added at the beginning of the extraction. After washing each crude lipid extract by adding one-fifth volume of 0.2% HCl solution, methylation was carried out with 2 ml of redistilled methanol, containing 2% H_2SO_4, under N_2 gas at 60°C over night (*1*). The methyl esters were separated by GLC (10% EGSS-X coated on Gaschrom P, column temperature 180°C) using a Yanaco G 180 gas chromatograph. Identification of each peak was made based on both relative retention time and carbon number as described by Hofstetter *et al.* (*10*).

The data were analyzed by a one-way analysis of variance and tested for significance at the $p < 0.05$ level by Fisher's method. Control group-associated comparisons were subjected to Student's *t*-test analysis.

RESULTS AND DISCUSSION

1. Dietary MCT influence on lipogenic enzyme activity (Experiment 1)

The effects of the various fats tested on liver lipogenic enzyme activity are

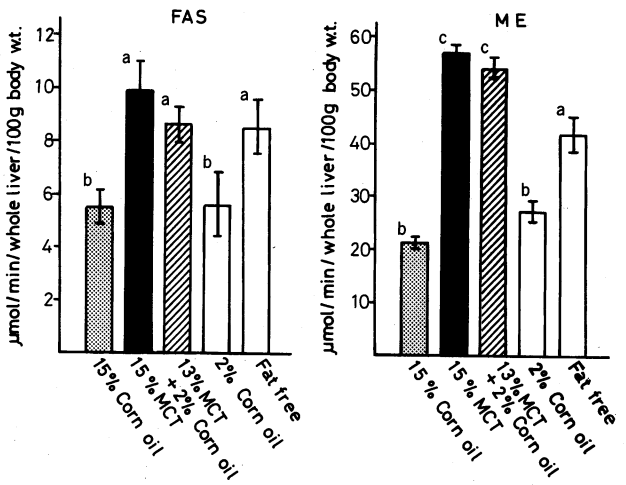

Fig. 1. Effect of dietary MCT on lipogenic enzyme activities in livers of rats refed for 7 days following 2-day fasting. Each bar represents the mean ± SEM of six rats. FAS and ME indicate fatty acid synthetase and malic enzyme, respectively. The enzyme activities are shown in μmol/whole liver/100 g body weight. Values between the bars with different superscripts are significantly different at $p < 0.05$. Similar results were obtained in specific activities of these enzymes.

shown in Table 2. The MCT-supplemented diet significantly increased FAS and ME activities. Feeding the tripalmitin or beef tallow diets did not lead to a marked elevation of these enzyme activities as compared to those in animals fed the corn oil diet. Tripalmitin added to the diet is reportedly absorbed at low levels (*11*), so the lack of enhancement in the FAS and ME activities of the tripalmitin group might have resulted in part from its low absorbability. However, it was evident that the beef tallow diet had no effect to increase the enzyme activities.

Meanwhile, as the MCT, tripalmitin or beef tallow diets each contained 2% corn oil, a linoleate inhibition of lipogenesis (*12*) might have coexisted to about the same extent among these three groups. The consumption of carbohydrate per 100 g body weight during an experimental period of 11 weeks was identical among all groups (Table 2). Thus, the increase in FAS and ME activities observed is possibly a specific effect of dietary MCT and not due to an increase in carbohydrate intake.

2. MCT increased linoleate requirement for inhibiting lipogenic enzyme activity (Experiment 2)

As shown in Fig. 1, the FAS and ME activities responded to a short-term (7-day) treatment with dietary MCT in a way similar to the long-term (11-week) treatment. The control groups demonstrated that the addition of 2% corn oil to the fat-free diet reduced FAS and ME activities. Meanwhile, adding 2% corn oil to the MCT-supplemented diet did not reduce the activities of FAS and ME, since the

activities remained nearly the same as in animals fed the 15% MCT diet. The 15% MCT diet markedly stimulated these enzyme activities compared to the 15% corn oil diet (Fig. 1). These high enzyme activity responses of the 15% MCT group could be attributed to two factors, one being the lack of EFA (*12*) and the other the MCT feeding itself.

The results of experiment 2 (Fig. 1) clearly suggest the possibility that 2% corn oil is not sufficient to dampen liver lipogenic enzyme activities in MCT-fed animals and that MCT feeding probably requires more EFA to inhibit the enzyme activity than diets free of MCT.

3. MCT effect on lipogenic enzyme activity is concentration dependent

The last experiment was conducted to ascertain whether or not the observed increase in enzyme activity was caused by MCT feeding itself. Feeding rats various amounts of dietary MCT (from 0 to 15% by weight) resulted in a proportional

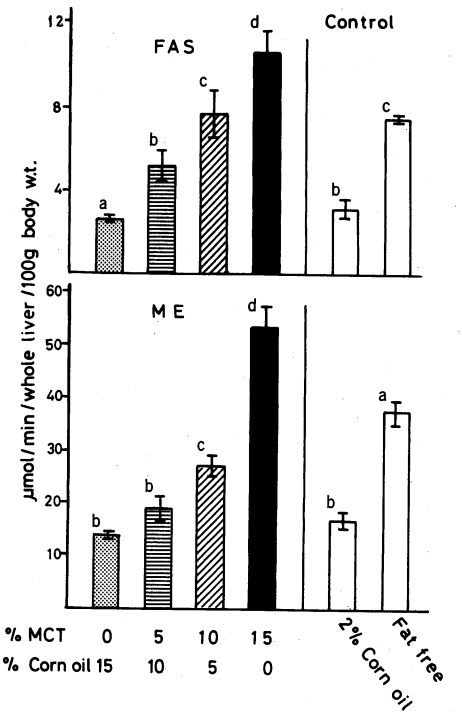

Fig. 2. Lipogenic enzyme activities in livers of rats refed on diets containing various amounts of dietary MCT. Each bar represents the mean ± SEM of six rats. FAS and ME indicate fatty acid synthetase and malic enzyme, respectively. The enzyme activities are shown in μmol/whole liver/100 g body weight. Values between the bars with different superscripts are significantly different at $p < 0.05$. Similar results were obtained in specific activities of these enzymes.

enhancement of FAS and ME activities in the livers, as shown in Fig. 2. The extent of elevated enzyme activities was dependent on the concentration of MCT in the total dietary fat.

On the other hand, the diets employed in this series of studies consisted of increasing amounts of MCT accompanied by a reduction in corn oil content. A question may arise as to whether the observed MCT effect in the dose-response study is due to MCT inclusion or to the accompanying reduction in corn oil, *i.e.*, the decreased availability of the inhibitory effect of corn oil. However, the answer can be found in the results from the control animals fed the 2% corn oil and fat-free diets. Namely, feeding rats the 2% corn oil diet significantly decreased FAS and ME activity in comparison with animals fed the fat-free diet. The extent of this decrease was 60% in both enzyme activities. On the other hand, replacing 5% MCT of the 15% MCT diet with corn oil provided about a 30% lower activity in FAS, whereas replacing 10% MCT of the 15% MCT diet with corn oil resulted in a 50% lower activity of FAS than the value for the 15% MCT group. The extent of the inhibitory effect of corn oil was not greater in animals fed the 10% corn oil + 5% MCT diet (about 2 g corn oil/100 kcal diet) than in animals fed the 2% corn oil diet (about 0.6 g corn oil/100 kcal diet). It was evident that when MCT was the prominent source of dietary fat, FAS and ME activities were certainly elevated. Therefore, the enhanced lipogenic enzyme activity must be mainly dependent on the amounts of MCT in the diet and not due to a reduction of dietary corn oil content. This result shows that MCT feeding would induce an increase in lipogenic enzymes.

4. Increase of mono-unsaturated fatty acids in liver lipids upon MCT feeding

The fatty acid composition in total liver lipids was observed in the same specimens as assayed the lipogenic enzyme activity in experiment 3 (Fig. 2). This (Table 3) revealed that the greater the dietary MCT content in the mixture of fat with corn oil was, the higher the levels of palmitic acid (16:0) and mono-unsaturated fatty acid (16:1ω7 and 18:1ω9) were. There was no appearance of 5,8,11-eicosatrienoic acid (20:3ω9) in the MCT-fed rats, except in the 15% MCT group. Therefore, it is conceivable that this phenomenon observed in the same animals in which the lipogenic enzyme activities were proportional to the concentration of dietary MCT (Fig. 2) was not induced by an EFA deficiency. The finding that the total level of 16:1ω7 and 18:1ω9 acids were raised as the dietary MCT content increased must be a consequence of accelerated fatty acid synthesis (*12,13*), possibly brought about by MCT ingestion itself.

Furthermore, the presence of a sufficient amount of corn oil, with more MCT existing in the diet (10% MCT + 5% corn oil group), provided a higher ratio (1.88) of $\omega 7+\omega 9/\omega 3+\omega 6$ acids than the ratio (0.85) obtained with the 2% corn oil diet. This is attributable to the fact that simultaneous with the increase in mono-unsaturated fatty acids, the total level of ω3 and ω6 acids in this group was less than that in the 2% corn oil group. But it is not clear that a greater amount of MCT in the mixed fat might lead to a reduction of the EFA level in liver, as the fatty acid

Table 3. Fatty acid composition in total liver lipids of rats refed on diets containing various amounts of dietary MCT.[1]

Fatty acids	Experimental groups (MCT : Corn oil)				Control	
	0 : 15	5 : 10	10 : 5	15 : 0	2% Corn oil	Fat-free
	Percent of total fatty acids					
14 : 0	0.5 ± 0.08[2,bc]	1.0 ± 0.2[ac]	1.3 ± 0.1[c]	1.1 ± 0.1[ac]	0.5 ± 0.02[b]	0.8 ± 0.1[a]
16 : 0	21.8 ± 0.7[b]	25.9 ± 1.8[ab]	33.5 ± 0.4[ac]	33.9 ± 1.3[c]	25.4 ± 2.8[ab]	29.1 ± 1.1[a]
16 : 1 (ω7)	1.9 ± 0.2[d]	4.6 ± 1.0[c]	7.4 ± 0.6[ab]	7.4 ± 0.5[b]	5.6 ± 1.6[bc]	9.4 ± 0.6[a]
18 : 0	14.7 ± 0.8[b]	12.8 ± 1.3[b]	11.5 ± 1.0[b]	12.7 ± 1.2[ab]	15.7 ± 1.9[b]	8.9 ± 0.6[a]
18 : 1 (ω9)	22.0 ± 0.6[bd]	26.5 ± 2.6[bc]	27.7 ± 2.0[c]	30.3 ± 1.5[ac]	21.2 ± 0.8[b]	36.2 ± 1.1[a]
18 : 2 (ω6)	17.3 ± 0.6[d]	11.5 ± 1.1[b]	6.0 ± 0.4[c]	2.0 ± 0.3[a]	11.4 ± 0.8[b]	2.7 ± 0.3[a]
20 : 3 (ω9)	—	—	—	0.6 ± 0.2[a]	—	0.4 ± 0.1[a]
20 : 4 (ω6)	17.3 ± 0.7[b]	14.3 ± 2.2[bc]	10.3 ± 1.3[ac]	8.4 ± 1.2[a]	15.3 ± 1.6[b]	9.0 ± 1.1[ac]
20 : 5 (ω3)	0.5 ± 0.1[a]	0.2 ± 0.1[a]	—	—	0.4 ± 0.04[a]	—
22 : 5 (ω6)	1.0 ± 0.1[cd]	0.7 ± 0.2[bc]	0.4 ± 0.1[ab]	0.1 ± 0.03[a]	0.5 ± 0.3[abd]	0.1 ± 0.02[a]
22 : 6 (ω3)	2.8 ± 0.3[ac]	2.5 ± 0.5[ac]	2.0 ± 0.2[ab]	3.5 ± 0.5[ab]	3.9 ± 0.3[b]	3.4 ± 0.7[ab]
ω7 + ω9	23.9	31.1	35.1	38.3	26.8	46.0
ω3 + ω6	38.9	29.2	18.7	14.0	31.5	15.2
ω7 + ω9/ω3 + ω6	0.61	1.07	1.88	2.74	0.85	3.0

[1] Data expressed as mean ± SEM of six rats. [2] Within a row, values not sharing common superscript letter are significantly different ($p < 0.05$).

composition was presented in the percentage of total fatty acids.

The 15% MCT group, because of its lack of EFA, showed a similar fatty acid composition to that of the fat-free group. These two groups showed a characteristic pattern of EFA deficiency (*5,14*), *i.e.*, not only was a significantly lower proportion of ω6-series acids (18 : 2ω6 and 20 : 4ω6) found in the liver lipids, but a significantly higher proportion of ω9-series acids (18 : 1ω9 and 20 : 3ω9) was also observed.

Further study is needed to explore the mechanism(s) by which MCT induces the high activity of lipogenic enzyme activity in rat liver.

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