

Effect of Corn Oil Feeding on Triglyceride Synthesis in the Rat

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ABSTRACT When rats were fed diets containing 10% corn oil for 2 weeks, α -glycerophosphate acyltransferase and diacylglycerol acyltransferase levels were reduced to 75% of that of controls fed a 0.5% corn oil diet, while glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase levels were reduced to 28, 33 and 66%, respectively. The incorporation of labeled glycerol or palmitic acid into triglycerides by liver slices was also reduced by corn oil feeding. Therefore, it is suggested that although the major reduction of triglyceride synthesis by linoleic acid feeding is due to fatty acid synthesis, glycerolipid synthesis is also reduced. *J. Nutr.* 110: 1138-1143, 1980.

INDEXING KEY WORDS corn oil · α -glycerophosphate acyltransferase · phosphatidate phosphatase · diacylglycerol acyltransferase

Although the control mechanism responsible for long chain fatty acid biosynthesis has been extensively studied (1), little information is available concerning the control of the syntheses of triglycerides and phospholipids from fatty acids. It is considered that when fatty acid synthesis is elevated, triglyceride synthesis becomes predominant. This view is supported by the studies of Ontoko (2) and of Sundler et al. (3) with isolated rat hepatocytes and by Rose et al. (4) with rat liver slices. It is also suggested that the triglyceride synthesis pathway was less affected by dietary manipulation than was the fatty acid synthesis pathway (5, 6). However, in rats fed a high-carbohydrate diet, the enzyme activities relating to triglyceride synthesis were markedly increased (7-10). A high-carbohydrate diet also increased the incorporation of [14 C]glycerol into hepatic triglyceride in the intact rat as well as in liver homogenate (7). It is also reported that α -glycerophosphate acyltransferase and phosphatidate phosphatase were increased by the high-fructose or glucose diet in the studies using liver microsomal and supernatant preparations (9); phosphatidate phosphatase activity seemed to be impor-

tant in the regulation of endogenous triglyceride formation under a high-carbohydrate diet (11). However, the factors which regulate the overall rate of liver triglyceride formation are not yet clear.

When rats were fed diets containing linoleic acids-rich oil such as corn oil, the activities of liver acetyl-CoA carboxylase, malic enzyme and glucose-6-phosphate dehydrogenase were lowered (12). Triglyceride formation may also be influenced by dietary corn oil, as in the case of a high-carbohydrate diet. In the present study, the effects of corn oil feeding on glycerolipid synthesis were investigated.

MATERIALS AND METHODS

Male Wistar rats, 5 weeks old (110-115 g), were fed sucrose diets for 2 weeks. The sucrose diets contained 60% sucrose, 18% casein, 6.9% cellulose, 0.5-10% corn oil (filling up to the 10% total with partially hydrogenated beef tallow), 5% salt mixture and 0.1% choline chloride and vitamin mixture. The fatty acid compositions of corn oil were 13.4% palmitic acid, 4.5% stearic acid, 39.2% oleic acid, 40.4% lino-

Received for publication 30 October 1979.

leic acid, and 2.5% others; those of partially hydrogenated beef tallow ("partially" was omitted below.) were 3.7% miristic acid, 17.8% palmitic acid, 53.9% stearic acid, 22.9% oleic acid and 1.7% others. The salt mixture contained: (%) CaCO_3 , 29.29; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, 0.43, KH_2PO_4 , 34.31; NaCl , 25.06; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 9.98; $\text{Fe}(\text{C}_6\text{H}_5\text{O}_7) \cdot 6\text{H}_2\text{O}$, 0.623; CuSO_4 , 0.156; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.121; ZnCl_2 , 0.020; KI , 0.0005; $(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.0025, and $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$, 0.0015. The vitamin mix furnished per 100 g diet of ration: retinyl palmitate, 2,000 IU; (mg) cholecalciferol, 0.01; DL- α -tocopherol, 40; thiamin·HCl, 1.0; riboflavin, 1.0; pyridoxine, 1.0; nicotinic acid, 8.0; folic acid, 0.25; calcium pantothenate, 2; biotin, 0.5; myo-inositol, 4.0, and menadione, 0.25.

The rat consumed 10–20 g diet/day during the experimental period. The rats were maintained in standard laboratory conditions (24°, automatic lighting schedule from 0700 to 1900 hours) and were paired with water ad libitum. After 2 weeks, rats were killed by decapitation between 1000 and 1100 hours. Blood was taken into test tube. The liver was quickly removed

to measure lipid synthesis by liver slices. The enzyme activities of fatty acid synthesis were measured in the 105,000 × g supernatant, as were the activities of glycerolipid synthesis in the microsomal fraction (between 10,000 × g and 105,000 × g) of liver homogenates.

Triglyceride and phospholipid syntheses by liver slices were determined as follows. Liver slices (250 mg) were incubated at 37° for 60 minutes with constant shaking of 2.5 ml of Krebs-Ringer phosphate buffer (Ca^{++} omitted), pH 7.4, containing additions as described for each experiment in tables 1 and 2. Fatty acids were used as bovine serum albumin complexes, which were prepared according to Spector and Hoak (13). Incubation was stopped by the addition of 20 ml of chloroform-methanol (2:1). Lipids were extracted according to Folch et al. (14) and separated by thin-layer chromatography on silica gel H, impregnated with 10 mM Na_2CO_3 . The lipids were developed with diethyl ether-benzene-acetic acid-ethanol (50: 40: 0.2: 2) for the separation of triglycerides and phospholipids. The lipids were identified by comparison with authentic standards, which

TABLE 1

Effects of dietary corn oil on the incorporation of labeled glycerol into triglycerides and phospholipids by liver slices in rats¹

Group	Body wt	Additions in incubation medium			
		0.08 mM Glycerol 0.08 mM Palmitic acid	0.08 mM Glycerol 0.08 mM Palmitic acid	0.08 mM Glycerol 0.08 mM Palmitic acid 0.08 mM Linoleic acid	0.08 mM Glycerol 0.08 mM Palmitic acid 0.08 mM Linoleic acid
Dietary corn oil	wt	Phospholipids	[¹⁴ C]Glycerol incorporation		Triglycerides
%	g		Triglycerides	Phospholipids	Triglycerides
			cpm × 10 ⁻³ per incubation		
0.5	165 ± 11	2.37 ± 0.39	3.45 ± 0.41	3.18 ± 0.22	4.45 ± 0.13
2.0	160 ± 7	2.84 ± 0.32	2.44 ± 0.21 ^a		
5.0	159 ± 9	2.81 ± 0.22	2.35 ± 0.27 ^a	2.84 ± 0.24	2.48 ± 0.46 ^b
10	170 ± 12	2.60 ± 0.39	1.91 ± 0.05 ^{b,c,d}	2.91 ± 0.15	2.04 ± 0.21 ^b

¹ Results expressed as mean ± SD (5–6 rats). The rats were fed for 2 weeks 10% fat diets, containing 0.5–10% corn oil with a filler of hydrogenated beef tallow. The fresh liver slices (250 mg) were incubated for 60 minutes at 37° during constant shaking in 2.5 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 0.08 mM [1(3)-¹⁴C]glycerol (The Radiochemical Centre, 0.125 μCi), 0.08 mM palmitic acid and 0.08 mM linoleic acid as indicated. Fatty acids were added as bovine serum albumin complex. Incorporations of the radioactive precursor into lipid fractions were determined after thin layer chromatography. Significantly different from 0.5% corn oil group, ^a $P < 0.01$, ^b $P < 0.001$. ^c Significantly different from 5% corn oil group at $P < 0.01$. ^d Significantly different from 2% corn oil group at $P < 0.001$. Significance of differences was calculated by Student's *t*-test.

TABLE 2
Effect of dietary corn oil on the incorporation of labeled palmitic acid into triglycerides and phospholipids by liver slices¹

Group Dietary corn oil	[¹⁴ C]palmitic acid incorporation	
	Phospholipids	Triglycerides
%	<i>cpm × 10⁻³ per incubation</i>	
0.5	1.77 ± 0.66	2.31 ± 0.36
5.0	1.88 ± 0.15	1.82 ± 0.34
10	1.89 ± 0.15	1.44 ± 0.16*

¹ Mean ± SD (five to six rats). Experimental conditions were the same as described for table 1, except that [U-¹⁴C]palmitic acid (0.05 μCi) was added as the radioactive precursor. Each incubation mixture contained 0.08 mM glycerol and 0.08 mM palmitic acid. * Significantly different from 0.5% corn oil group at *P* < 0.01.

were visualized by exposure to iodine vapor. The silica gel zone corresponding to each lipid fraction was scraped into a counting vial, and the radioactivity was determined in Bray's solution (15) by a liquid scintillation counter (Model 3385, Packard Instruments, Downers Grove, IL).

The assay of α-glycerophosphate acyltransferase (EC 2.3.1.15) was based on the method of Brandes et al. (16) with modifications. The incubation mixture contained 0.06 M Tris-HCl buffer, pH 7.4, 0.03 mM palmityl-CoA and 0.5 mM L-[U-¹⁴C]α-glycerophosphate (0.05–0.1 μCi, New England Nuclear, Boston, MA). The mixture was pre-warmed to 37° and the incubation was initiated by the addition of 50–100 μg of liver microsomes in 0.35 ml and carried out for 2 minutes at 37°. The reaction was stopped by the addition of 4 ml chloroform-methanol (2:1). After acidification the lipids were extracted and then again extracted with 4 ml of methanol:0.1 N HCl (1:1) three times. The radioactivities of the lipids were determined as above.

Diacylglycerol acyltransferase (EC 2.3.1.20) was assayed as follows. The incubation mixture (0.2 ml) contained 50 mM dithiothreitol, 5.4 mM MgCl₂, 0.3 mM palmityl-CoA (1,000 cpm/nmol), 4 mM 1,2-diacylglycerol (derived from egg yolk phosphatidylcholine), 0.2 mg Tween 80, 1.75 mg fatty acid free bovine serum al-

bumin and 150 mM Tris-HCl buffer, pH 7.5. The mixture was pre-warmed at 37° and then the reaction was initiated by adding 50–100 μg microsomes. The mixture was incubated with shaking at 37° for 15 minutes; the reaction was terminated by adding 5 ml of a mixture of petroleum ether:isopropanol:1 N H₂SO₄ (10:40:1). Thirty minutes later, 4 ml petroleum ether and 2 ml water were added and the mixture was shaken vigorously. 2 ml of 0.2 M KHCO₃ and 2 ml of petroleum ether were added to the upper phase, and the mixture was again shaken vigorously. The upper phase was collected and the lower phase was extracted again with 1 ml petroleum ether. The two petroleum ether extracts were combined and washed with 1.5 ml 1 M NaCl. The extract was separated by thin-layer chromatography on silica gel H (Merck) impregnated with 10 mM Na₂CO₃, using ether:hexane:acetic acid (25:75:1). The silica gel zone corresponding to triglycerides was scraped into a counting vial, and the radioactivities were determined.

Phosphatidate phosphatase (EC 3.1.3.4) was assayed as described by Lamb et al. (11). Microsomes were incubated in 35 mM Tris-malate buffer (0.6 ml), pH 6.5, at 37° for 30 minutes and the release of inorganic phosphate in the presence and absence of a 2 mM aqueous dispersion of phosphatidate was measured. The reaction was stopped by the addition of 0.4 ml of 5% trichloroacetic acid. After cooling, the precipitate was removed by centrifugation. The amount of inorganic phosphate in the supernatant was measured by molybdenum method essentially according to Berenblum et al. (17).

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was assayed according to Glock and McLean (18), and malic enzyme (EC 1.1.1.40) according to Ochoa (19). Acetyl-CoA carboxylase activity (EC 6.4.1.2) was assayed by the [¹⁴C]HCO₃⁻ fixation method (20). Triglycerides were measured according to Fletcher (21).

All enzyme activities were assayed at 37° and expressed as nanomoles of products synthesized or substrates used per minute per milligram of cytosol or microsomal protein (table 3). Protein concentra-

tion was determined by the method of Lowry et al. (22).

RESULTS AND DISCUSSION

After the rats were fed for 2 weeks the 10% fat diets containing 0.5, 2, 5 and 10% corn oil (filling up to the 10% total with hydrogenated beef tallow), the body weights were not significantly different among the groups, as shown in table 1. Therefore, it is suggested that hydrogenated beef tallow gave growth comparable to corn oil.

When liver slices were incubated with radioactive glycerol, the incorporation of glycerol into triglycerides was decreased in the groups fed the diets containing more than 2% corn oil compared with 0.5% corn oil diet group, as shown in table 1. Since unsaturated fatty acids are linked preferentially to the C-2 position of triglyceride, linoleic acid as well as palmitic acid were added in the incubation medium. However, no effect of linoleic acid addition was found on incorporation of glycerol into triglycerides.

When liver slices were incubated with radioactive palmitic acid, the incorporation

of palmitic acid into triglycerides was also decreased in the 5 or 10% corn oil group, as compared with that of the 0.5% corn oil control as shown in table 2. In contrast to triglyceride synthesis, phospholipid synthesis from glycerol and palmitic acid occurred at virtually constant rates regardless of the amount of dietary corn oil. Previously we found that phospholipid synthesis occurred at a constant rate in dietary-induced alterations of fasted and refed rats (6). The present results seem to support the previous hypothesis that the synthesis of triglycerides and that of phospholipids are controlled independently. The activities of hepatic α -glycerophosphate acyltransferase and diacylglycerol transferase were significantly decreased by feeding 5% and 2% corn oil diet, respectively (table 3). The enzyme activities were correlated with incorporation of glycerol or palmitic acid into triglycerides by liver slices in vitro study. On the other hand, Wiegand et al. (23) reported that the activities of hepatic glycerophosphate acyltransferase in rats fed for 1 week diets containing 2.5–15% safflower oil were not significantly different from those of rats fed diets containing the

TABLE 3

Effect of dietary corn oil on enzyme activities of fatty acid and triglyceride synthesis¹

Group	Glucose-6-phosphate dehydrogenase ²	Malic enzyme ²	Acetyl-CoA carboxylase ²
Dietary corn oil			
%		nmoles/min/mg (37°)	
0.5	397 ± 37	324 ± 21	28.8 ± 2.2
5.0	133 ± 28 ^c	160 ± 22 ^c	20.6 ± 1.1 ^c
10	113 ± 10 ^c	106 ± 10 ^{c, *}	19.0 ± 1.3 ^c

Group	α -Glycerophosphate acyltransferase ²	Diacylglycerol acyltransferase ²	Phosphatidate phosphatase ²
Dietary corn oil			
%		nmoles/min/mg (37°)	
0.5	0.59 ± 0.02	5.59 ± 0.34	0.60 ± 0.09
2.0	0.57 ± 0.03	4.61 ± 0.52 ^b	
5.0	0.43 ± 0.10 ^{b, d}	4.40 ± 0.30 ^c	0.63 ± 0.01
10	0.44 ± 0.10 ^{b, d}	4.21 ± 0.32 ^c	0.66 ± 0.06

¹ Mean ± SD (five rats). Rats were fed for 2 weeks on 10% fat diet as described in table 1. ² Enzymes were assayed as described in "Materials and Methods." Enzyme activities were expressed as nmoles substrate utilized or ² product synthesized per minute per milligram of cytosol (upper column) or microsomal (lower column) protein. Significantly different from 0.5% corn oil group, ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$. ^d Significantly different from 2% corn oil group at $P < 0.05$. ^{*} Significantly different from 5% corn oil group at $P < 0.001$ (by *t*-test).

TABLE 4
Effect of dietary corn oil on triglycerides level¹

Group Dietary corn oil	Triglycerides	
	Serum	Liver
%	mg/ml	mg/g
0.5	2.54 ± 0.43	20.9 ± 3.31
5	2.25 ± 0.44	14.9 ± 2.40 ^b
10	1.99 ± 0.11 ^a	12.2 ± 2.56 ^c

¹ Mean ± SD (five to six rats). Animals were the same described in table 1. Significantly different from 0.5% corn oil group, ^a $P < 0.05$, ^b $P < 0.02$; ^c $P < 0.01$ (by *t* test).

corresponding amounts of cocoa butter. The discrepancy of the results might be due to the differences of the strain and age of rats, the kinds of dietary linoleic acid-rich oil and control fat, and also the feeding period. Lamb et al. (9, 11) suggested that microsomal and supernatant phosphatidate phosphatase is important in the regulation of endogenous triglyceride formation. However, the microsomal enzyme activity was not changed by increasing the amount of dietary corn oil in this experiment.

Although microsomal activities of α -glycerophosphate acyltransferase (in the common step of triglyceride and phospholipid syntheses) were decreased to 75% of control by corn oil feeding, phospholipid syntheses by liver slices were not decreased. Some factors that control phospholipid synthesis at a constant rate may exist in liver slices. It was reported that a binding protein for long chain fatty acids in the cytosol of liver, called Z protein, activated diacylglycerol transferase (24). Likewise, the fractions other than microsomes may be involved in glycerolipid synthesis of microsomal enzymes in dietary manipulation. On the other hand, it seems reasonable to postulate that triglyceride and phospholipid syntheses are regulated after diacylglycerol step rather than during the common steps.

By feeding the 10% corn oil diet, glucose-6-phosphate dehydrogenase, malic enzyme and acetyl-CoA carboxylase levels were reduced to 28, 33 and 66%, respectively, compared with the 0.5% corn oil group. α -Glycerophosphate acyltransferase

and diacylglycerol acyltransferase were reduced to 75%. Consequently, serum and liver triglyceride levels were reduced by increasing dietary corn oil, as shown in table 4. In this study, it was found that although the enzymes of fatty acid synthesis were more effectively reduced by feeding corn oil than were the enzymes of glycerolipid synthesis, the enzymes of glycerolipid synthesis were also reduced to some extent by increasing dietary corn oil.

LITERATURE CITED

1. Numa, S. & Yamashita, S. (1974) Current Topics in Cellular Regulation (Horecker, B. L. & Stadtman, E. R., eds.), vol. 8, pp. 197-248, Academic Press, New York.
2. Ontko, J. A. (1972) Metabolism of free fatty acids in isolated liver cells. *J. Biol. Chem.* 247, 1788-1800.
3. Sundler, R., Akesson, B. & Nilsson, A. (1974) Effect of different fatty acids on glycerolipid synthesis in isolated rat hepatocytes. *J. Biol. Chem.* 249, 5102-5107.
4. Rose, H., Vaughan, M. & Steinberg, D. (1964) Utilization of fatty acids by rat liver slices as a function of medium concentration. *Am. J. Physiol.* 206, 345-350.
5. Nishikori, K., Iritani, N. & Numa, S. (1973) Levels of acetyl coenzyme A carboxylase and its effectors in rat liver after short-term fat-free refeeding. *FEBS Lett.* 32, 19-21.
6. Iritani, N., Yamashita, S. & Numa, S. (1976) Dietary control of triglyceride and phospholipid synthesis in rat liver slices. *J. Biochem.* 80, 217-222.
7. Waddell, M. & Fallon, H. J. (1973) The effect of high-carbohydrate diets on liver triglyceride formation in the rat. *J. Clin. Invest.* 52, 2725-2731.
8. Fallon, H. J. & Kemp, E. L. (1968) Effects of diet on hepatic triglyceride synthesis. *J. Clin. Invest.* 47, 712-719.
9. Lamb, R. G. & Fallon, H. J. (1974) An enzymatic explanation for dietary induced alterations in hepatic glycerolipid metabolism. *Biochim. Biophys. Acta* 348, 179-188.
10. Fallon, H. J., Barwick, J., Lamb, R. G. & van den Bosch, H. (1975) Studies of rat liver microsomal diglyceride acyltransferase and choline phosphotransferase using microsomal-bound substrate: effects of high fructose intake. *J. Lipid Res.* 16, 107-115.
11. Lamb, R. G. & Fallon, H. J. (1974) Glycerolipid formation from sn-glycerol-3-phosphate by rat liver cell fractions: The role of phosphatidate phosphohydrolase. *Biochim. Biophys. Acta* 348, 166-178.
12. Muto, Y. & Gibson, D. M. (1970) Selective dampening of lipogenic enzymes of liver by exogenous polyunsaturated fatty acids. *Biochem. Biophys. Res. Commun.* 38, 9-15.

13. Spector, A. A. & Hoak, J. C. (1969) An improved method for the addition of long-chain free fatty acid to protein solutions. *Anal. Biochem.* **32**, 297-302.
14. Folch, J., Lees, N. & Sloane-Stanley, G. H. (1957) A simple method of the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.
15. Bray, G. A. (1960) A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**, 279-285.
16. Brandes, R., Olley, J. & Shapiro, B. (1963) Assay of glycerol phosphate acyltransferase in liver particles. *Biochem. J.* **86**, 244-247.
17. Berenblum, I. & Chain, E. (1938) An improved method for the colorimetric determination of phosphate. *Biochem. J.* **32**, 295-298.
18. Glock, G. E. & McLean, P. (1953) Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. *Biochem. J.* **55**, 400-408.
19. Ochoa, S. (1955) Malic enzyme. *Meth. Enzymol.* **1**, 739-753.
20. Nakanishi, S. & Numa, S. (1970) Purification of rat liver acetyl coenzyme A carboxylase and immunochemical studies on its synthesis and degradation. *Eur. J. Biochem.* **16**, 161-173.
21. Fletcher, M. J. (1968) A colorimetric method for estimating serum triglyceride. *Clin. Chim. Acta* **22**, 393-397.
22. Lowry, O. H., Rosenborough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275.
23. Wiegand, R. D., Rao, G. A. & Reiser, R. (1973) Dietary regulation of fatty acid synthetase and microsomal glycerophosphate acyltransferase activities in liver. *J. Nutr.* **103**, 1414-1424.
24. O'Doherty, P. J. A. & Kuksis, A. (1975) Stimulation of triacylglycerol synthesis by Z protein in rat liver and intestinal mucosa. *FEBS Lett.* **60**, 256-258.