Effects of Diet on Hepatic Triglyceride Synthesis

HAROLD J. FALLON and E. LEON KEMP

From the Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

ABSTRACT The effects of diet on the rate of triglyceride synthesis by rat liver homogenates was measured. Changes in triglyceride synthesis were correlated with the level of activity of L-α-glycerophosphate acyltransferase, the enzyme catalyzing the first specific reaction in hepatic glycerolipid synthesis.

Fasting for 48–72 hr depressed the synthesis of triglyceride from L-α-glycerophosphate. High carbohydrate diets, fed to rats for 6 days, resulted in increased triglyceride synthesis. Diets high in starch were less effective than high glucose, sucrose, or fructose diets in increasing triglyceride synthesis. Diets high in corn oil did not alter triglyceride synthesis. These studies established the importance of dietary factors in the regulation of hepatic triglyceride synthesis.

L-α-Glycerophosphate acyltransferase activity was measured after the same dietary changes. Both high carbohydrate and high fat diets resulted in increased enzyme specific activity. Fasting for 72 hr did not decrease activity. Thus, the specific activity of this enzyme did not correlate well with the measured rate of triglyceride synthesis indicating that other factors must participate in the regulation of triglyceride biosynthesis.

INTRODUCTION

The regulation of hepatic fatty acid synthesis has been studied by several investigators (1–3). However, relatively little is known of the factors that regulate the hepatic synthesis of glycerolipids, including triglycerides and phospholipids. The liver is the major source of plasma triglycerides exclusive of chylomicrons. The factors that control hepatic triglyceride synthesis may contribute to the etiology of endogenous or carbohydrate-induced hyperlipemia (4–6), the hyperlipemia of diabetes mellitus (7), and some forms of fatty infiltration of the liver. The effetcs of several dietary changes on the rate of triglyceride synthesis by liver homogenates are reported in these studies.

The first specific reaction in endogenous glycerolipid synthesis is catalyzed by the enzyme L-α-glycerophosphate acyltransferase [GPAT (Fig. 1)]. This reaction is common to the formation of many phospholipids and glycerides. The enzyme may be measured in liver by the L-α-glycerophosphate–dependent release of coenzyme A (CoA) from palmityl CoA. Previous investigators have suggested that the rate of esterification of L-α-glycerophosphate may be important in the regulation of fatty acid synthesis (8–10). Therefore, dietary effects on the activity or level of this enzyme in liver might be associated with changes in both fatty acid and glycerolipid synthesis.

In these studies, the activity of hepatic GPAT was correlated with the rate of triglyceride synthesis under various dietary conditions. In addition, the in vitro effects of changes in substrate and albumin concentration on reaction velocity were recorded.

METHODS

Materials. Palmityl CoA was prepared from palmityl chloride by the method of Seubert (11). The product was identified spectrophotometrically as described by Srere, Seubert, and Lynen (12). The purity was determined by ultraviolet absorption at 260 mμ and hydroxamate forma-

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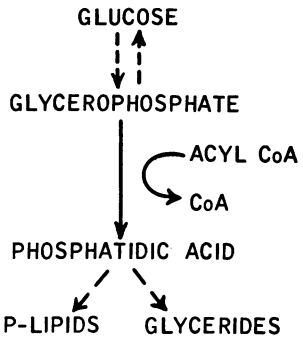

FIGURE 1 The de novo pathway of glycerolipid formation in liver. L-α-Glycerophosphate, derived from glycerol or carbohydrate intermediates, is esterified with long-chain acyl CoA derivatives by the microsomal enzyme, L-α-glycerophosphate acyltransferase.

tion (13). Palmityl CoA was stored at −4°C in H_2O at pH 5 or dry, under N_2.

D,L-Glycerol-3-phosphate, and palmityl chloride were obtained from Sigma Chemical Co., St. Louis, Mo. Fatty acid–poor albumin was purchased from Pentex, Inc., Kankakee, Ill., and human fraction III-0 (β-lipoprotein) from Hyland Laboratories, Los Angeles, Calif. Dithiothreitol, D,L-α-tocopherol, and high protein casein were obtained from Calbiochem, Los Angeles, Calif. All lipid standards were purchased from Applied Science Laboratories Inc., State College, Pa.

L-Glycerol-3-phosphate-^{14}C was obtained from International Chemical and Nuclear Corporation, City of Industry, Calif. or Nuclear Research Chemicals Inc., Orlando, Fla. and identified chromatographically.

Preparation of microsomes. Rats were decapitated, and their livers removed and placed in 4 volumes of iced 0.25 M sucrose containing 0.05 M Tris-HCl, pH 6.5, and 0.02 mM D,L-α-tocopherol, hereafter called sucrose-Tris buffer. The liver was homogenized and the homogenate successively centrifuged at 600 *g* for 10 min, twice at 15,700 *g* for 15 min, and once at 105,000 *g* for 30 min, in refrigerated centrifuges. The last supernate was decanted and the residue resuspended in an equal volume of fresh sucrose-Tris buffer and centrifuged at 105,000 *g* for 30 min. The supernate was again discarded and the microsomal pellet suspended in a volume of sucrose-Tris buffer equal to one-fourth the original homogenate volume. Microsomes were stored in stoppered containers at 4°C for 48–72 hr. The identity of this fraction was verified by electron microscopy.[1]

Enzyme assays. The assay of GPAT was based on the method of Brandes, Olley, and Shapiro (14) with several modifications. The assay mixture contained 0.08 M Tris-HCl buffer, pH 6.5, 0.01 M dithiothreitol, 0.4–0.6 mM palmityl CoA, 0.06 M D,L-α-glycerophosphate, and 0.01 ml of microsomal preparation (0.2–1.0 mg/ml of protein) in a final volume of 0.34 ml. Assays were performed in the presence of optimum concentrations of albumin (7.5 mg/ml). Duplicate assays were conducted in the presence and absence of D,L-α-glycerophosphate. Microsomes were added after a 5 min preincubation at 30°C and the reaction stopped after a 15 min incubation at 30°C by addition of 3.5% (w/v) perchloric acid. The mixture was centrifuged to remove the perchlorate-insoluble palmityl CoA. The release of soluble CoA was measured spectrophotometrically at 260 mμ with molar extinction coefficient (*E*) of 16,000. The increased release of CoA in the presence of D,L-α-glycerophosphate was a measure of GPAT activity. The concentration of palmityl CoA varied as noted in the tables, which accounts for some differences in control GPAT activity between experiments. All determinations were made in duplicate. Units of activity are expressed as change in optical density per 15 min. All results recorded are means ± SD.

The incorporation of L-α-glycerophosphate-^{14}C into lipids by microsomal fractions was measured under conditions identical to those noted above. The L-α-glycerophosphate-^{14}C (28 μc/μmole) was added in a volume of 0.02 ml containing 600,000 cpm. The reaction was stopped by adding 20 volumes of chloroform-methanol (2:1 v/v), and lipids were extracted by the method of Folch, Lees, and Sloane-Stanley (15). The total radioactivity in the lipid extract was measured by liquid scintillation spectroscopy.

Rat liver homogenates were prepared in 4 volumes of 0.25 M sucrose containing 0.01 M Tris-HCl buffer, pH 7.5. The homogenate was centrifuged (800 *g*) at 4°C for 10 min and the resulting supernatant used in further studies. The homogenate (0.2 ml) was incubated for 15 min at 37°C with 10 mM Tris-KCl, pH 7.5, 3 mM potassium phosphate buffer, pH 7.5, 3 mM $MgCl_2$, 3 mM ATP, 0.5 mM dithiothreitol, 4 mM coenzyme A, 1 mM Na palmitate, 5 mg of albumin, and 0.35–0.65 μc of L-α-glycerophosphate-^{14}C in a total volume of 0.7 ml. The reaction was stopped by the addition of 13.3 ml of chloroform-methanol (2:1 v/v). Lipids were extracted, redissolved in benzene, and individual glycerides and phospholipids separated by thin-layer chromatography. Most of the radioactivity was recovered in the triglyceride fraction under the conditions of this assay. Radioactivity of samples isolated by thin-layer chromatography was determined by liquid scintillation spectroscopy. Activity is expressed in counts per minute converted to lipid per microgram of homogenate protein per 15 min.

Total liver triglyceride was determined by the dichromate reduction method (16) after separation by thin-layer chromatography in the solvent system *n*-hexane–diethyl ether–glacial acetic acid (73:25:2). L-α-Glycerophosphate was determined in liver by the method of Nikkila and Ojala (17). Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (18). Microsomes were digested with 1 M NaOH before protein measurement.

Animals and diets. Male Sprague-Dawley rats (weight 150–250 g) were used in all studies. Rats were individually caged and killed without fasting by decapitation. The high glucose diet contained (by weight) 24% casein, 64% glucose, 4% Vitamin Fortification Mix (Nutritional

[1] Performed by Dr. Fredrick Dalldorf.

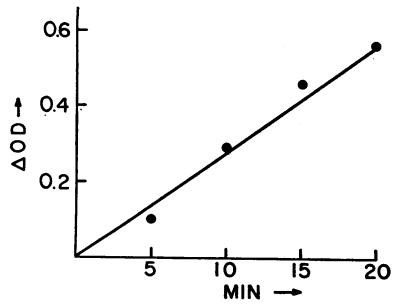

FIGURE 2 The release of CoA from palmityl CoA during a 20 min time period. The time course for L-α-glycerophosphate acyltransferase (GPAT) measurement by the assay described is shown to be linear for a 20 min period. The difference in optical density (ΔOD) between assays conducted with and without L-α-glycerophosphate is recorded on the ordinate for each time period.

Biochemicals Corporation, Cleveland, Ohio) and 4% Hegsted salt mixture. The high corn oil diet contained 240 g of casein, 40 g of salt mixture, 40 g of vitamin mixture, 90 g of glucose, and 284 g of corn oil/700 g of diet. An isocaloric amount (0.7 g) of this diet was fed to the high corn oil group for every 1 g of control diet consumed by the pair-fed controls. Control rats were fed Purina Laboratory Chow containing 23% protein, 4.5% fat, and 52% carbohydrate. The carbohydrate was composed of mixed starches and less than 5% cane molasses. Rats were pair fed isocaloric amounts of each diet for 6 days. Weight gain was similar for each group of rats.

RESULTS

Assay of L-α-glycerophosphate acyltransferase. The linear increase in release of CoA during a 20 min time period is shown in Fig. 2. All determinations were performed within this time period. The relationship of activity to the concentration of microsomes is shown in Fig. 3. There was a linear increase in CoA release when the microsomal concentration was increased from 0.1 to 1.5 mg of protein/ml of incubation mixture. Higher concentrations of microsomes regularly reduced enzyme activity. Therefore, microsomal preparations were diluted to an appropriate protein concentration before assay.

Omission of the sulfhydryl reagent, dithiothreitol, reduced activity approximately 20%. No increase in enzyme activity was observed upon the addition of up to 6 mmoles/liter of EDTA-2 Na, $MgCl_2$, or $CaCl_2$. Details of the effects of other additions will be reported elsewhere.[2]

The assay of GPAT activity by measurement of CoA release was correlated with the simultaneous incorporation of L-α-glycerophosphate-^{14}C into lipids (Fig. 4). Other studies have shown a linear relationship of these two measurements over a fivefold range of microsomal concentration.[2] The mean ratio of CoA release to L-α-glycerophosphate incorporation was 1.1. A similar ratio of 1.3 was previously reported by Stansly (19). Failure to obtain the theoretical ratio of 2 might be attributed to utilization, binding, or destruction of the free CoA released during the 15 min incubation. However, no decrease in CoA concentration was observed when an equivalent amount of CoA was incubated with microsomes and palmitate in the absence of palmityl CoA under assay conditions.

More likely explanations would be a positional specificity in the acylation of L-α-glycerophosphate (20) or a possible sequential acylation of L-α-glycerophosphate as proposed by Lands and Hart (21). A major product of the reaction would then be a monoacyl glycerophosphate and would account for the observed ratio of less than 2. This possibility is supported by finding the predominant radioactivity in a chromatographic spot tentatively identified as monoacyl glycerophosphate.[2] The remainder of radioactivity is found in phosphatidic acid.

Effects of substrate and albumin concentration. The increase in GPAT reaction rate with increasing concentration of L-α-glycerophosphate is shown in Fig. 5. The physiological range of L-α-glycero-

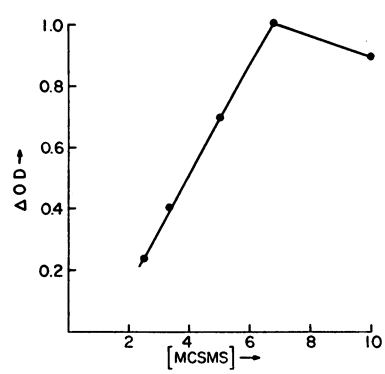

FIGURE 3 The effect of increasing microsomal concentration on GPAT activity. The scale of microsomal concentration ([*MCSMS*]) is adjusted so that 10 is equivalent to 0.7 mg of microsomal protein per assay (2.0 mg/ml). The decrease in GPAT activity at high microsomal concentration was a reproducible effect regardless of rat age or diet.

[2] Fallon, H. J., and R. Lamb. Manuscript in preparation.

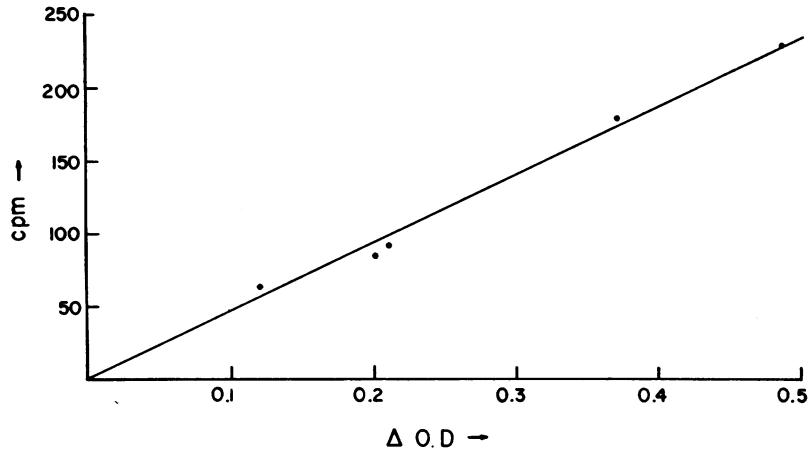

FIGURE 4 The relationship of CoA released to L-α-glycerophosphate-^{14}C incorporation. The linear relationship of CoA release (ΔOD) as measured spectrophotometrically to incorporation of L-α-glycerophosphate-^{14}C (cpm) into total lipids is shown. This linear relationship has been demonstrated for the complete range of GPAT activity observed in these studies. The ratio of CoA release to L-α-glycerophosphate incorporation has varied from 0.98 to 1.20.

phosphate (8, 22) in liver is indicated by the arrows. Alterations in L-α-glycerophosphate concentration as reported in various dietary (8, 17, and Table I) or hormonal states (20) may result in significant changes in the rate of this reaction in liver. A similar relationship exists for the other substrate of the reaction, palmityl CoA (Fig. 6). The physiological range of long-chain acyl CoA content in liver (8) is shown in Fig. 6. It is apparent that changes in long-chain acyl CoA content may also be associated with significant alterations in GPAT reaction rate.

The activating effect of albumin on this reaction is shown in Fig. 7. Bovine albumin in Tris buffer, pH 6.5, was added to the reaction mixture until maximum reaction rates were achieved. At concentrations of albumin higher than 8 mg/ml, the activating effect gradually diminished. A similar effect of albumin has been noted previously in studies of microsomal lipid synthesis (22). The mechanism of this effect of albumin is under further investigation. Human serum β-lipoprotein in the same concentrations could substitute for albumin, but other serum proteins were inactive.

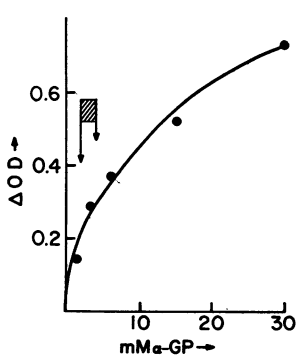

FIGURE 5 The effect of increasing L-α-glycerophosphate concentration on GPAT reaction rate. The physiological range for L-α-glycerophosphate concentration is shown by the arrows. GPAT activity is recorded as in Fig. 2. A tentative Michaelis constant, K_m, for L-α-glycerophosphate may be calculated from these data, namely, $K_m = 6.7 \times 10^{-3}$ M.

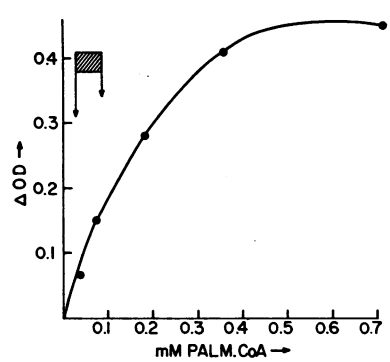

FIGURE 6 The effect of increasing palmityl CoA concentration on GPAT reaction rate. The physiological range for long-chain acyl CoA derivatives in liver is shown by the arrows. GPAT activity is recorded as in Fig. 2. A tentative Michaelis constant for palmityl CoA is $K_m = 1.1 \times 10^{-4}$ M.

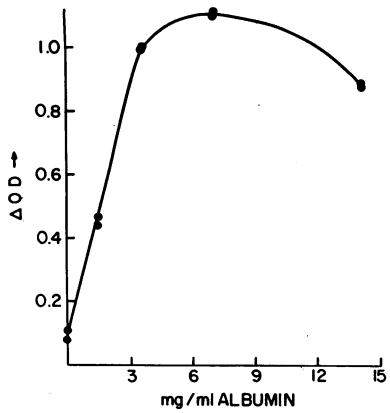

FIGURE 7 The effect of albumin concentration on GPAT. Increasing albumin concentration in the assay mixture resulted in increased enzyme activity as measured by CoA release until concentrations of 7 mg/ml or higher were attained. Standard assays contained 4 mg/ml of albumin.

The maximum rate of L-α-glycerophosphate esterification by GPAT under optimum in vitro conditions was estimated to be 3.45 μmoles/g of liver per hr. A similar calculation for triglyceride synthesis from L-α-glycerophosphate-^{14}C by liver homogenates was 1.85 μmoles/g of liver per hr. Since the substrate concentrations for GPAT in vivo are always significantly less than those used to measure the maximum activity in vitro, it is likely that the reaction velocity of GPAT in liver is significantly less than the maximum rate.

Effects of diet. The effects of fasting on endogenous lipid synthesis were studied and are shown in Table I. Fasting for 48 hr and 72 hr produced no change in the GPAT specific activity (defined here as activity per milligram of microsomal protein). However, a 46% depression in the total GPAT activity in the livers of fasted rats was noted, an effect largely attributable to a decrease in total liver weight. Total GPAT activity was 97.4 ± 34.9 U per liver in the fed rats and 52.9 ± 7.9 U per liver in the fasted group, a significant difference ($P < 0.01$).

The liver concentration of L-α-glycerophosphate was also shown to decline after 72 hr of fasting (Table I), which confirms previous observations (9). Fasting for 48 or 72 hr resulted in a marked depression in the over-all rate of triglyceride synthesis as measured by the incorporation of L-α-glycerophosphate-^{14}C into triglyceride by liver homogenates. A similar decrease in L-α-glycerophosphate incorporation into lecithin and monoacyl glycerophosphate occurred during fasting.

TABLE I

Effects of Fasting on Hepatic Glyceride Synthesis

	GPAT	L-α-Glycerophosphate	GP-^{14}C into triglyceride
	U/mg protein ± SD	*μmoles/g wet wt* ± SD	*cpm/μg protein* ± SD
Control	1.87 ± 0.23	3.60 ± 0.45	1.48 ± 0.41
48-hr fast	2.18 ± 0.48	2.48 ± 0.11*	0.11 ± 0.08*
72-hr fast	2.16 ± 0.38	2.07 ± 0.46*	0.15 ± 0.09*

Five rats were studied in each group. The concentration of palmityl CoA in the GPAT assay was 0.6 mmole/liter. Methods are described in the text.
* Differences from control significant ($P < 0.01$).

The effects of diets containing 64% glucose on hepatic triglyceride synthesis are shown in Table II. The rate of incorporation of L-α-glycerophosphate into triglyceride was doubled by this diet when compared to rats fed chow. An 80% increase in hepatic GPAT activity was noted in the rats fed high glucose diets, but no change was noted in either L-α-glycerophosphate or triglyceride content.

Further studies have shown that high carbohydrate diets containing 64% fructose, 64% potato starch, or 32% sucrose and 32% starch result in increased triglyceride synthesis and GPAT activity (Table III). The diet containing 64% starch was significantly less effective than the other carbohydrates in increasing the rate of L-α-glycerophosphate incorporation into triglycerides.

TABLE II

Liver GPAT Activity and Triglyceride Synthesis in Rats Fed High Glucose and High Corn Oil Diets

Diet	GPAT	L-α-Glycerophosphate	GP-^{14}C into triglycerides
	U/mg protein ± SD	*μmoles/g wet wt* ± SD	*cpm/μg protein* ± SD
Control	1.74 ± 0.5*	4.2 ± 0.1	0.55 ± 0.2
Glucose	2.92 ± 0.4‡	3.7 ± 0.7	1.03 ± 0.3‡
Corn oil	3.53 ± 0.3‡	3.9 ± 0.4	0.65 ± 0.3

All assays were conducted as described in Table I except that the palmityl CoA concentration for the GPAT assay was 0.5 mmole/liter. Six rats were used in each group.
* Mean value for six rats fasted 44 hr was 1.76 ± 0.4.
‡ Value significantly different from control group, $P < 0.01$.

TABLE III

Hepatic GPAT Activity and Triglyceride Synthesis in Rats Fed High Carbohydrate Diets

Diet carbohydrate	GPAT	GP-^{14}C into triglycerides
	U/mg protein ± SD	*cpm/μg protein* ± SD
Control	1.38 ± 0.14	0.86 ± 0.43
Glucose	2.18 ± 0.42	4.43 ± 2.10
Fructose	2.42 ± 0.15	4.73 ± 1.84
Starch	2.32 ± 0.39	2.58 ± 1.20
Starch-sucrose	2.09 ± 0.49	5.52 ± 1.67

Six rats were fed the appropriate diet for 7 days. The control animals were fed chow and pair fed with rats fed glucose. All groups gained weight at approximately the same rate. All experimental diets contained 64% carbohydrate. The mixed starch-sucrose diet contained 32% starch and 32% glucose. The starch used was potato starch. The palmityl CoA concentration used in the GPAT assay was 0.45 mmole/liter. The differences between control values and those for rats fed high carbohydrate diets were all significant ($P < 0.01$) for both GPAT assay and the incorporation of L-α-glycerophosphate-^{14}C into triglyceride. The increase in triglyceride synthesis in rats fed a 64% starch diet was significantly less than for other carbohydrates ($P < 0.01$).

Rats fed diets containing 40% corn oil showed significantly higher levels of GPAT activity than those fed high glucose diets [$P < 0.01$ (Table II)]. However, no significant increase in incorporation of L-α-glycerophosphate-^{14}C into triglyceride or lecithin was observed in these animals. Incorporation of L-α-glycerophosphate into other phospholipid fractions has not been measured. The hepatic concentration of L-α-glycerophosphate was unchanged by the 40% corn oil diet. A marked increase in liver total triglyceride was observed in the rats fed corn oil. The mean total triglyceride for this group was 32.3 mg/g wet weight in comparison with 4.6 mg/g in the glucose fed rats and 3.8 mg/g in the control rats. Similar increases in the triglyceride content of liver of rats fed high fat diets has been observed previously (23).

DISCUSSION

The measurement of GPAT in rat liver has been shown to be reproducible and linear with respect to time and microsomal concentration. The assay method used correlated well with the measurement of GPAT reaction rate with labeled substrate. The maximum activity of GPAT in normal rat liver was not in great excess of the simultaneously measured rate of triglyceride synthesis. This finding would suggest that hepatic GPAT activity is a potential rate-limiting reaction, and that changes in enzyme activity or content could contribute to the regulation of glycerolipid synthesis.

Dietary-induced changes in substrate concentration for GPAT might alter the rate of this reaction in vivo. The concentrations of substrates for GPAT, L-α-glycerophosphate, and long-chain acyl CoA derivatives have been measured in liver (8, 17) and are considerably lower than the levels which gave maximum enzyme velocity in vitro. This observation suggests that physiological changes in the concentration of these substrates may result in corresponding variations in the rate of lipid synthesis by changing the reaction velocity of GPAT. For example, the depression in L-α-glycerophosphate concentration noted during prolonged fasting could contribute to the observed decrease in the rate of triglyceride synthesis. Further observations on the effects of diet, hormones, and drugs on the concentration of these substrates may elucidate the possible importance of this form of regulation in lipid biosynthesis.

Changes in the specific activity of GPAT under various dietary conditions were observed. However, these changes correlated poorly with the rate of hepatic triglyceride synthesis after fasting and high fat feeding as measured by isotopic tracer techniques. Therefore, the importance of changes in enzyme level to the regulation of triglyceride synthesis remains uncertain. The relation of hepatic GPAT levels to the rate of synthesis of other glycerolipids has not been evaluated.

The effect of fasting on triglyceride synthesis was profound. The observed depression may be explained in part by the decrease in L-α-glycerophosphate concentration, but it is likely that other factors contribute to this effect. Fatty acid synthesis has been reported to decline in the fasted animal (24). Thus several factors may contribute to depressed triglyceride synthesis during fasting and contribute to a diversion of carbohydrate intermediates from lipid synthesis to other pathways.

High carbohydrate diets have been shown repeatedly to result in increased serum triglyceride levels in man (4–6). This effect is apparently exaggerated in many patients with carbohydrate-induced hypertriglyceridemia (7). Increased en-

dogenous synthesis of triglyceride is the presumed cause of the hypertriglyceridemia (7).

The observed increase in hepatic triglyceride synthesis by liver homogenates from rats fed various high carbohydrate diets is evidence that an increased endogenous production of triglyceride does occur during carbohydrate feeding. Previous studies have shown an increase in fatty acid synthesis (24) and L-α-glycerophosphate dehydrogenase (25) during high carbohydrate feeding. These factors and the observed rise in hepatic GPAT levels may contribute to the increased triglyceride synthesis during high carbohydrate ingestion. The increased synthesis does not result in triglyceride accumulation in the liver.

The probable physiological importance of the observed twofold increase in the rate of endogenous triglyceride synthesis is suggested by the limited rate of triglyceride removal from plasma (26). Increased triglyceride synthesis in the absence of fatty liver would be expected to result in hypertriglyceridemia when the capacity for removal from plasma is exceeded.

The effect of 40% corn oil feeding on glyceride synthesis is apparently more complex. High fat diets suppress fatty acid synthesis (27) but appear to simultaneously increase the specific activity of hepatic GPAT. This increase in enzyme activity was not accompanied by an increase in endogenous triglyceride synthesis from L-α-glycerophosphate. There was no increased incorporation of L-α-glycerophosphate into lecithin in the rats fed 40% corn oil.

The high fat diet, unlike the high glucose diet, resulted in a fatty liver and possibly interfered with the release of lipid from the liver. The factors regulating lipid synthesis during high fat ingestion are currently under further study.

These studies have demonstrated that changes in the capacity of liver tissue to synthesize triglyceride in vitro are produced by alterations in diet. The dietary content of carbohydrate seems most important in mediating these changes. Further studies will be necessary to fully describe the mechanism of the carbohydrate-induced changes in the rate of hepatic glyceride production in vivo.

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