Regulation of triglyceride biosynthesis in adipose and intestinal tissue

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Abstract The synthesis of phosphatidic acid and di- and triglycerides via the glycerol-3-phosphate pathway is markedly inhibited by 2-monooleyl ether in microsomal and whole cell preparations obtained from adipose and intestinal tissue. Monoglycerides are also inhibitors under conditions in which their hydrolysis is minimized. A correlation between inhibition by, and the hydrolysis of, monoglycerides has been demonstrated. 2-Monooleyl ether is the most effective inhibitor of the several mono- and di- ethers and esters studied. The specificity of the inhibition of glycerol-3-phosphate acylation by 2-monoethers or 2-monoesters has been demonstrated because microsomal NADH– and NADPH–cytochrome *c* reductase activities were not significantly inhibited. The reported control mechanism for triglyceride biosynthesis is discussed in relation to the regulation of fatty acid uptake and release in adipose tissue and the absorption and metabolism of triglycerides by the intestinal mucosa.

Supplementary key words monoglyceride and glycerol-3-phosphate pathways · 2-monoethers

The synthesis of triglycerides in most tissues occurs primarily via the glycerol-3-phosphate pathway, with the exception of the intestinal mucosa, which utilizes predominately the monoglyceride pathway (1). Recently, the monoglyceride reactions have also been demonstrated in adipose tissue (2, 3). When the glycerol-3-phosphate pathways in liver, adipose tissue, and intestinal mucosa were compared, it was shown that the specific enzymatic activity of the microsomal fraction from each of these tissues was similar (2). However, 80–90% of the triglyceride biosynthesized occurs via the monoglyceride pathway in the intestinal mucosa if monoglyceride is available (1). Several suggestions have been offered to explain these observations. The first suggestion was based on the kinetic properties of the two enzyme systems; it was shown that the K_m for the monoglyceride pathway was lower than that for the glycerol-3-phosphate pathway (4). It has also been suggested that the monoglyceride pathway can be specifically activated by bile salts (5).

In the course of our investigations on the comparative study of the glycerol-3-phosphate and monoglyceride pathways in adipose tissue, a significant decrease in the incorporation of labeled glycerol-3-phosphate into higher glycerides was noted when 2-monooleyl ether was added (6). In the present investigations these initial observations have been further documented in relation to enzyme and substrate specificity of the inhibition. A similar control mechanism is also described for intestinal tissue.

MATERIALS AND METHODS

Racemic disodium glycerol-3-phosphate and cytochrome *c* were obtained from Sigma Chemical Co., St. Louis, Mo. NADPH, NADH, and ATP were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Palmitoyl CoA and CoA were obtained from P-L Biochemicals, Milwaukee, Wis. The albumin (fraction V) and collagenase were obtained from Armour Pharmaceuticals, Chicago, Ill., and Worthington Biochemical Corp., Freehold, N.J., respectively. The ATP was dissolved in 0.25 M Tris–maleate and adjusted to pH 7.0. [U-^{14}C]Glycerol-3-phosphate and [U-^{14}C]glucose were obtained from New England Nuclear Corp., Boston, Mass. The 2-octadecenyl [9,10-^{3}H]glycerol ether, referred to as 2-monooleyl ether, was synthesized via the procedure of Wood and Snyder (7). The radiopurity

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TABLE 1. Effect of 2-monooleyl ether on the incorporation of glycerol-3-phosphate into phosphatidic acid using adipose tissue microsomes

2-Monooleyl ether added (nmoles)	0	100	500	1000	2000
[U-^{14}C]Glycerol-3-phosphate utilization (pmoles/min/mg protein)	6895	6256	4774	4750	3185
P values[a]		NS[b]	NS	<0.05	<0.01

The flasks contained 10 μmoles of [U-^{14}C]glycerol-3-phosphate, 50 μmoles of $MgCl_2$, 150 μmoles of KCl, 1 μmole of palmitoyl CoA, 0.1 ml of 10% Tween 80, 0.5 ml of microsomes in 0.154 M KCl, and 0.5 M Tris–maleate buffer (pH 7.0). 1 mg of microsomal protein in a total volume of 2.0 ml was incubated at 20°C for 30 min. The results shown are the averages of four experiments.

[a] P values compared with control values without 2-monooleyl ether were calculated according to paired t test.

[b] NS, not significant.

was determined by thin-layer chromatography, employing silica gel H in a developing solvent of chloroform–methanol 98:3. The nonlabeled 2-monooleyl ether was synthesized in our laboratory or obtained from Serdary Research Laboratories, London, Ontario, Canada. The 2-monooleyl ether has been shown to be a substrate for the monoglyceride pathway in the intestinal mucosa and adipose tissue (2, 8, 9). The advantage of using the ether is that acyl migration or hydrolysis of the side chain cannot occur. The 2-octadecenoyl [U-^{14}C]-glycerol, referred to as 2-monoolein, was synthesized in our laboratory (10) and further purified on silica gel impregnated with boric acid (11). The nonlabeled 2-monoolein was kindly supplied by Dr. Fred H. Mattson, Procter & Gamble Co., Cincinnati, Ohio. The sodium taurodeoxycholate (grade A) was obtained from Calbiochem, San Diego, Calif., and its purity was checked by thin-layer chromatography for conjugated bile acids according to the procedure of Hofmann (12).

Tissue and substrate preparation

All tissues were obtained from male golden hamsters that had been fed ad lib. The intestinal microsomes were prepared via the procedure previously described (13, 14). The adipose tissue microsomes were isolated from a homogenate prepared in a 0.25 M sucrose medium as previously described (2). The isolation procedure, using a defatted homogenate, was similar to that of Roncari and Hollenberg (15), with the exception that the sucrose medium was substituted for 0.15 M KCl. The isolated adipose cells were prepared via the method of Rodbell (16) as modified by Schotz et al. (17). Protein concentration was determined by the method of Lowry et al. (18). Intestinal rings were prepared and incubated in micellar solutions according to previously reported procedures (19), with the exception that higher concentrations of monoethers and fatty acids were employed as indicated. The albumin–glyceride complex was prepared by dissolving the glyceride in diethyl ether and then adding defatted albumin (20) dissolved in 0.5 M Tris–maleate buffer, pH 7.0. The diethyl ether was removed under vacuum, and the albumin–glyceride complex was sonicated for 10 sec at maximum power (Bronson Sonic Power Co., Plainview, N.Y.).

Enzyme assays

After incubation the tissues were extracted, and the neutral lipids, fatty acids, and phosphatidic acids were quantitated (2, 21, 22). Phosphatidic acids were the major product synthesized via the glycerol-3-phosphate pathway when microsomes were used (4). Two additional microsomal enzyme systems were assayed. The enzymes NADH– (EC 1.6.2.2) and NADPH–cytochrome c reductase (EC 1.6.2.3) were assayed according to the procedure of Masters, Williams, and Kamin (23). The reduction of cytochrome c was followed in the recording spectrophotometer (550 nm) in a reaction mixture containing 0.1 ml of sample, cytochrome c (3.6×10^{-5} M), 0.05 M potassium phosphate buffer, pH 7.7, containing 1×10^{-4} M EDTA, KCN (10^{-3} M), and either NADPH or NADH (1×10^{-4} M) in a final volume of 1.1 ml. The enzymatic activity was calculated from the initial linear rates.

RESULTS

Inhibition using adipose tissue microsomes

The results obtained when adipose tissue microsomes were incubated with increasing concentrations of 2-monooleyl ether are given in Table 1. The incorporation of [U-^{14}C]glycerol-3-phosphate into phosphatidic acid was determined. All values have been corrected for radioactivity incorporated into phosphatidic acids in the absence of palmitoyl CoA, and the data presented are the averages of four experiments. The reported experimental procedure was modified in three additional ways in order to establish the specificity of the monoether inhibition. None of the modifications significantly affected the inhibition by the monoether shown in Table 1. Albumin was substituted for Tween 80. Albumin will bind the 2-monooleyl ether to facilitate solubilization. These experiments demonstrated that Tween 80 was not responsible for the inhibition. Secondly, since the inhibitor also utilizes palmitoyl CoA as a substrate, it was determined whether the palmitoyl CoA concentration was limiting. Therefore, various concentrations of this substrate (0.25–1.5 mM) were used. In addition, [1-^{14}C]palmitoyl CoA was utilized at various concentrations and the unreacted radioactive compound was determined at the end of the incubation. Under both

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TABLE 2. Effect of 2-monooleyl ether on the glycerol-3-phosphate pathway in isolated fat cells

2-Monooleyl ether added (nmoles)	0	250	500	1000	2000
Glyceride-glycerol formed from [U-^{14}C]glucose (nmoles/100 mg wet wt)	75	73	72	62	56
P values		NS	NS	<0.05	<0.01

Each flask contained 3% albumin in Krebs-Ringer-bicarbonate buffer (pH 7.4) and 3.0 mM [U-^{14}C]glucose. The 2-monooleyl ether and 2 ml of isolated fat cells were added in bicarbonate buffer. The final volume was 2.5 ml. The incubation was for 1 hr at 37°C in a 95% O_2–5% CO_2 atmosphere. The results shown are the averages of five experiments.

conditions it was demonstrated that the palmitoyl CoA was not limiting. Therefore, the inhibition could not be explained by the fact that the 2-monooleyl ether was competing with glycerol-3-phosphate for the available acyl CoA. Finally, the fatty acid activation system was substituted for palmitoyl CoA in the incubation to see whether the detergent properties of palmitoyl CoA could explain these results. Palmitic acid was added as the potassium salt at the same concentration as the palmitoyl CoA. Since no significant differences in the inhibition were observed by these modifications, it was concluded that the 2-monooleyl ether was responsible for the observed inhibition.

Inhibition with adipocytes

In order to determine whether the inhibition could be demonstrated at a higher level of organization, intact adipose cells were prepared from epididymal fat pads. The 2-monooleyl ether was employed at a concentration range of 0–0.8 mM, and the glycerol-3-phosphate was synthesized from the added [U-^{14}C]glucose. The calculations were based on the synthesis of 2 moles of glycerol-3-phosphate formed via the glycolytic reactions per mole of glucose. Under these conditions approximately 90% of the ^{14}C radioactivity in the higher glycerides was found in the glycerol moiety of the synthesized di- and triglycerides. The results of these experiments are given in Table 2. As can be seen, increasing amounts of 2-monooleyl ether resulted in a decreased utilization of glucose for glyceride-glycerol formation. When the averages of five experiments were compared using the paired *t* test, the *P* value was < 0.01 for the flask containing 2 μmoles of 2-monooleyl ether.

Intestinal microsomes and the control of glyceride synthesis

The intestinal mucosa has also been shown to synthesize triglycerides via both the monoglyceride and glycerol-3-phosphate pathways (1). The monoglyceride pathway is the predominant reaction sequence involved in triglyceride biosynthesis in rat (24, 25), hamster (4, 26), and man (27). Previous investigations had suggested that one possible explanation for this preferential utilization of monoglycerides was the lower K_m of the enzyme complex (4). Although the concentration and the K_m values of the enzymes in these systems may be of importance in modifying each of the respective pathways, additional control mechanisms such as those described in adipose tissue may be present. To test this postulate, a series of experiments similar to those with adipose tissue microsomes was carried out using the intestinal mucosa as the enzyme source. Table 3 demonstrates the effect of increasing concentrations of the 2-monooleyl ether on the synthesis of higher glycerides via the glycerol-3-phosphate pathway. The major product synthesized under these conditions is phosphatidic acid (4). The reported results are corrected for the synthesis that occurred in the absence of ATP. As is shown, the synthesis of phosphatidic acid from glycerol-3-phosphate in the presence of increasing concentrations of the 2-monooleyl ether by intestinal microsomes is markedly inhibited.

TABLE 3. Effect of 2-monooleyl ether on the incorporation of glycerol-3-phosphate into phosphatidic acid using intestinal microsomes

2-Monooleyl ether added (nmoles)	0	100	500	2000
[U-^{14}C]Glycerol-3-phosphate utilization (pmoles/min/mg protein)	4300	3100	1900	720
P values		NS	<0.05	<0.01

The flasks contained 5 μmoles of L-[U-^{14}C]glycerol-3-phosphate, 1 μmole of potassium palmitate, 30 μmoles of ATP, 25 μmoles of KF, 10 μmoles of $MgCl_2$, 25 μmoles of GSH, 0.5 μmole of CoA, 6.4 mg of fatty acid-free bovine serum albumin in 0.4 ml of 0.5 M Tris-HCl buffer (pH 7.0), 0.4 ml of 0.5 M Tris-maleate buffer (pH 7.0), and 1 mg of microsomal protein in 0.154 M KCl. The total volume of 2.0 ml was incubated at 30°C in a Dubnoff bath for 30 min.

Intestinal rings and the control of glyceride biosynthesis

In order to investigate the possible physiological importance of the above results, the effect of 2-monooleyl ether on the glycerol-3-phosphate pathway was examined using intestinal slices as the enzyme source. Micelles containing taurodeoxycholate, oleate, glucose, and 2-monooleyl ether were prepared by the method previously reported (19), and labeled glucose was added to the incubation medium in order to follow the glycerol-3-phosphate pathway. It was demonstrated that over 90% of the ^{14}C radioactivity recovered in the di- and triglycerides was present in the glycerol portion of the molecule. Table 4 shows the effect of 2-monooleyl ether in these incubations. The experiment was per-

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TABLE 4. Effect of 2-monooleyl ether on the glycerol-3-phosphate pathway using intestinal slices

2-Monooleyl ether added (nmoles)	0	200	500	1000	2000
Glyceride-glycerol formed from [U-^{14}C]glucose (nmoles/100 mg tissue)	29	24	20	7	4

The flasks contained 5.52 μmoles of sodium taurodeoxycholate and 1.84 μmoles of oleate in micellar form, 4.6 μmoles of [U-^{14}C]-glucose in Krebs-Ringer phosphate buffer (minus Ca^{2+} and Mg^{2+}, pH 6.3), and the 2-monooleyl ether. The final volume of 2.3 ml was incubated at 37°C in an atmosphere of 100% O_2 for 30 min. The averages of two experiments are shown. No statistical analyses were performed.

formed twice and the results were similar; no statistical analysis was performed. The results shown are the average values obtained for two experiments. Increasing amounts of 2-monooleyl ether in the micellar solution caused a progressive inhibition of [U-^{14}C]glucose incorporation into the glyceride-glycerol. Although the 2-monooleyl ether inhibits the glycerol-3-phosphate pathway, this compound is also a substrate for the monoglyceride pathway. Hence, it was important to determine the amount of unreacted 2-monooleyl ether. It was found that approximately 350 nmoles of the 2-monooleyl ether was utilized when either 500 or 2000 nmoles of the 2-monooleyl ether was included in the micelle incubation mixtures. Therefore, adequate quantities of the unreacted 2-monooleyl ether were present to account for the observed inhibition.

Specificity of inhibitors

The specificity of the inhibition was investigated using the more physiologically occurring monoglyceride as well as certain diglycerides. The experiment was performed twice, since limited quantities of some of the compounds tested were available. The results of such experiments using hamster intestinal and adipose microsomes are given in Table 5. The results are expressed in terms of percentage inhibition compared with the control flasks. 500 nmoles of each compound was used. With adipose tissue microsomes the inhibition was observed only when the 2-monooleyl ether was added. The corresponding esters were not inhibitory under similar conditions. When intestinal microsomes were used, a marked inhibition was demonstrated with both the monoethers and monoesters and to a very limited degree with the diglycerides.

Comparison of ethers and esters as inhibitors

As was previously discussed, one of the reasons for using the ether analog was its resistance to hydrolysis. An explanation for the observed differences in inhibition by monoglycerides between the intestinal mucosa and adipose tissue is a difference in the rate of hydrolysis of the ester. The monoglyceride-hydrolyzing enzyme (EC 3.1.1.3) was first reported in intestinal tissue (28) and more recently in adipose tissue (29). The monoethers and esters are also utilized in the biosynthetic reactions. In order to determine the relative effective concentration of monoethers and esters present during the incubation, radioactive 2-monooleyl ether and 2-monoolein were employed. The unreacted inhibitor and the biosynthetic and hydrolytic products were determined for both compounds. The results of this experiment are given in Table 6. As can be seen, a significant amount of the 2-monooleyl ether was present at the end of each incubation. When 2-monoolein was used with adipose tissue microsomes, the inhibitor was almost completely absent at the end of the incubation and no inhibition (8%) of the glycerol-3-phosphate pathway was observed. Furthermore, the synthesis of higher glycerides could be demonstrated in adipose tissue only when the ether analog was used (41 nmoles) because the ester analog was rapidly cleaved. These results are in contrast to those obtained with intestinal microsomes, in which a similar higher glyceride synthesis and inhibition of the glycerol-3-phosphate pathway was noted. As is also evident, a significant amount of the 2-monoolein remained at the end of the incubation with this tissue (130 nmoles). The failure of adipose tissue to use 2-monoolein as a substrate as well as it uses 2-monoethers for the biosynthesis of triglycerides and as an inhibitor of glycerol-3-phosphate utilization can be explained by its rapid hydrolysis. The subcellular distribution of the monoglyceride hydrolase and the activation of this enzyme are presently being investigated.

TABLE 5. Effect of various mono- and dioleyl ethers and esters on the glycerol-3-phosphate pathway using adipose and intestinal microsomes

	Inhibition of Glycerol-3-phosphate Utilization	
Additions[a]	Adipose Tissue	Intestinal Tissue
	%	
None	0	0
2-Monooleyl ether	43	77
1-Monooleyl ether	0	31
2-Monoolein	0	46
1-Monoolein	0	49
1,2-Diolein	2	22
1,3-Diolein	0	13

The incubation flasks contained 10 μmoles of [U-^{14}C]glycerol-3-phosphate, 150 μmoles of KCl, 10 μmoles of $MgCl_2$, 1 μmole of potassium palmitate, 30 μmoles of ATP, 0.5 μmole of CoA, 25 μmoles of KF, and 6.4 mg of fatty acid-free bovine serum albumin in 0.8 ml of 0.5 M Tris–maleate buffer (pH 7.0). 0.5 μmole of the inhibitor was suspended in 0.8 ml of the albumin solution. 1 mg of microsomal protein was added in a total volume of 2.0 ml. The incubation was at 25°C for 30 min. The averages of two experiments are shown.

[a] 500 nmoles of each compound was used.

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TABLE 6. Metabolic fate of 2-monoolein and 2-monooleyl ether using adipose and intestinal microsomes

	Inhibition of Glycerol-3-phosphate Utilization	Higher Glycerides	Unreacted Substrate Recovered
	%	nmoles	
Adipose tissue			
2-Monoolein	8	12	8
2-Monooleyl ether	28	41	443
Intestinal tissue			
2-Monoolein	54	50	130
2-Monooleyl ether	55	81	412

The incubation flasks contained 10 μmoles of glycerol-3-phosphate, 150 μmoles of KCl, 10 μmoles of $MgCl_2$, 1 μmole of potassium palmitate, 30 μmoles of ATP, 0.5 μmole of CoA, 25 μmoles of KF, and 6.4 mg of fatty acid-free bovine serum albumin in 0.8 ml of 0.5 M Tris–maleate buffer (pH 7:0). 0.5 μmole of either ^{3}H-labeled 2-monooleyl ether or [^{14}C]glycerol-labeled 2-monoolein was used. The inhibitors were suspended in 0.8 ml of the albumin solution. The percent inhibition was determined using [U-^{14}C]glycerol-3-phosphate. 1 mg of microsomal protein in a total volume of 2.0 ml was incubated at 25°C for 30 min.

TABLE 7. Specificity of 2-monooleyl ether inhibition of microsomal enzyme systems

Enzyme Assayed	Flask	Adipose Tissue	Intestinal Tissue
		nmoles/min/mg microsomal protein	
Phosphatidate synthesis	Control	2.1	2.6
	+ 2-MOE[a]	0.5	0.6
	P value	<0.05	<0.05
NADPH–cytochrome *c* reductase	Control	27.7	27.8
	+ 2-MOE	26.3	26.9
	P value	NS	NS
NADH–cytochrome *c* reductase	Control	333	418
	+ 2-MOE	282	375
	P value	NS	NS

The incubation flasks contained 10 μmoles of [U-^{14}C]glycerol-3-phosphate, 150 μmoles of KCl, 10 μmoles of $MgCl_2$, 1 μmole of potassium palmitate, 30 μmoles of ATP, 0.5 μmole of CoA, 25 μmoles of KF, and 6.4 mg of fatty acid-free bovine serum albumin in 0.8 ml of 0.5 M Tris–maleate buffer (pH 7.0). 2000 nmoles of 2-monooleyl ether was suspended in 0.8 ml of the albumin solution. The microsomal protein added per flask was 1.5 mg (adipose) or 1.2 mg (intestinal). A total volume of 2.0 ml was incubated at 20°C for 30 min. The data presented are the averages of three experiments.

[a] 2-Monooleyl ether.

Specificity of the inhibition of microsomal enzymes

The second question regarding the specificity of the inhibition was concerned with whether other microsomal enzymes may be inhibited by the 2-monoether or ester. To test this possibility, two different microsomal enzyme systems, NADH– and NADPH–cytochrome *c* reductase, in addition to the glycerol-3-phosphate pathway, were monitored in the presence of the 2-monooleyl ether. The results of these experiments employing adipose and intestinal microsomes are given in Table 7. This experiment has been repeated numerous times with no significant variation in the reported results. As can be seen, no significant inhibition in the activities of either of the reductase enzymes was observed in either tissue. In contrast, the glycerol-3-phosphate pathway was inhibited 66% and 75% in adipose and intestinal microsomes, respectively.

DISCUSSION

The present investigations established the inhibition of the glycerol-3-phosphate pathway by 2-monooleyl ether in adipose tissue and in the intestinal mucosa (Tables 1–4). The latter tissue appears to be even more sensitive to the control mechanism. Direct evidence was also obtained that suggested that the inhibition is relatively specific for monoglycerides or their ether analogs (Table 5) as well as for the acylation of glycerol-3-phosphate because two other microsomal enzyme systems were not affected by the addition of monooleyl ether (Table 7). A series of experiments was also carried out in an attempt to ascertain the type of inhibition involved. Although the data would be consistent with a competitive type of inhibition, reservation should be placed on this mechanism because the physical state of the inhibitor is difficult to determine. The specific enzymatic step involved in the inhibition would appear to be the acylation of glycerol-3-phosphate. This conclusion is based on the fact that no significant quantity of lysophosphatidic acid accumulates in the presence of the inhibitor.

Several recent observations have been reported that indicate that 2-monoglycerides may be important in the regulation of triglyceride biosynthesis in adipose tissue. The specificity of lipoprotein lipase for positions 1 and 3 of the triglyceride molecule suggests that the resulting monoglycerides may be important in the uptake of triglycerides in adipose tissue (30). This inhibition mechanism may be important in facilitating the release of fatty acids from adipose tissue by inhibiting their reincorporation into glycerides via the glycerol-3-phosphate pathway under conditions in which the hormone-sensitive lipase has been activated. The physiological function of monoglycerides in the biosynthesis of triglycerides has been well established in the intestinal mucosa. The functional role of monoglycerides in adipose tissue as a substrate for triglyceride biosynthesis and an inhibitor of the glycerol-3-phosphate pathway may be a question because the activity of the enzyme that hydrolytically cleaves monoglycerides is thought to be in excess (29). However, the activity of this enzyme has been reported only for broken cell preparations. Previous reports from our laboratory indicated that monoglycerides were utilized for higher glyceride synthesis

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directly when isolated fat cells were employed (2). If the monoglyceride hydrolase were a lysosomal enzyme, the difference in monoglyceride utilization in broken and whole cells might be explained. Investigations are now in progress to determine the subcellular distribution of this hydrolytic enzyme. The concentration of monoglycerides reported to be present in adipose tissue is 3.9 mM (31). In the reported study, a concentration of 0.25 mM of the 2-monooleyl ether inhibited the glycerol-3-phosphate pathway approximately 50%. Although it is difficult to extrapolate the concentration data presented in terms of physiological importance because factors such as subcellular compartmentalization must occur, the amount of the inhibitor required for activity is a physiological and not a pharmacological concentration. The demonstrated presence of the monoglyceride pathway in adipose tissue (2) in combination with reported inhibitory effects of monoglycerides on the glycerol-3-phosphate pathway suggests that this class of compounds may be important in modulating fatty acid release and triglyceride synthesis in this tissue. It has been reported that the biosynthesis of triglycerides is increased by the addition of certain lipolytic hormones (31), which would be in contrast to the predicted results based on the reported observations. Experiments are now in progress in an attempt to explain this discrepancy.

The reported mechanism for the regulation of the glycerol-3-phosphate pathway in triglyceride biosynthesis from monoglycerides in intestinal mucosa explains certain documented in vivo and in vitro observations at the enzyme level. Monoglycerides and fatty acids are known to enter through the intestinal mucosa cell during the process of digestion and absorption of triglycerides. These compounds are formed due to the specificity of pancreatic lipase (EC 3.1.1.3). As was discussed, the major pathway for triglyceride biosynthesis is the monoglyceride pathway in this tissue. The reported results offer convincing evidence to further explain these observations since the added monoglycerides are serving not only as a substrate for the biosynthesis of triglycerides but also as an inhibitor of synthesis by the glycerol-3-phosphate sequence when monoglycerides are available. Via this mechanism, the synthesis of triglycerides occurs predominately by the monoglyceride pathway. When the intracellular level of monoglyceride is decreased due to its biosynthetic utilization, the synthesis could now shift to the glycerol-3-phosphate pathway because the inhibition is decreased owing to the lower monoglyceride concentration. The reported studies on the intestinal mucosa provide a mechanism for the biosynthesis of triglyceride via the most efficient mechanism based on availability of substrate.

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