Synthesis and Accumulation of Triglycerides in Liver of Diabetic Rats
Effects of Insulin Treatment

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SUMMARY

The relationship between the severity of diabetes and the hepatic accumulation of triglycerides in the streptozotocin-diabetic rat was studied. A consistent increase in hepatic triglyceride content was observed only in the ketotic diabetic state. Insulin treatment of the ketotic diabetic rat resulted in the reversal of increased plasma concentrations of glucose, free fatty acids, β-hydroxybutyric acid, and triglycerides to those observed in nondiabetic controls. Insulin treatment of the ketotic rats, however, did not completely eliminate the accumulation of the hepatic triglycerides.

To determine whether increased triglyceride synthesis was a contributory factor to the hepatic triglyceride accumulation, triglyceride synthesis was studied in vitro using nuclear-free homogenates of liver. Triglyceride synthesis increased in ketotic diabetes. Insulin treatment of the ketotic rats partially reversed the increased diglyceride synthesis observed in ketosis but had no effect on the accelerated triglyceride synthesis. The effects of ketotic diabetes and insulin treatment of the diabetic rat on the activities of two key enzymes of the triglyceride biosynthetic pathway were examined next. The maximal velocities (V_{max}) of liver-soluble phosphatidate phosphohydrolase and of microsomal diglyceride acyltransferase increased in ketosis. Insulin treatment of the diabetic rats reverted the activity of these two enzymes to control values.

The results of our study demonstrate that hepatic triglyceride synthesis in ketotic rats accelerates at a time when hepatic triglyceride content increases. The increased synthesis of triglycerides was associated with increases in the activities of two key enzymes of triglyceride biosynthesis. There was a correlation between hepatic triglyceride content and triglyceride synthesis in control, ketotic diabetic, and insulin-treated rats. These observations suggest a physiologic role for the observed changes in the accumulation of hepatic triglycerides in ketotic diabetes. DIABETES 28:472–478, May 1979.

Hepatic triglycerides are known to accumulate in experimental diabetes.[1–3] The accumulation may result from increased synthesis of triglyceride (TG) or its decreased output from liver [as very low density lipoproteins (VLDL)] or a combination of these factors. VLDL released from liver is considered to be one measure of TG synthesis in liver, since the total output of TG by the liver is proportional to the uptake and esterification of free fatty acids (FFA).[4,5] Hepatic TG are complexed with the appropriate apoproteins and phospholipids before released as VLDL.[6] Reports on TG synthesis, measured as release of VLDL, in liver of diabetic animals are variable. Heimberg and colleagues[4,7,8] observed decreased secretion of TG in perfused livers of alloxan-diabetic rats. In the pancreatectomized dog with chronic diabetes, Basso and Havel[9] noted that the secretion of VLDL-TG was depressed in response to an infusion of palmitate. Balasse, Bier, and Havel,[10] however, observed increased synthesis and release of TG in dogs made diabetic abruptly with guinea pig anti-insulin serum. Spitzer et al.[11] did not observe differences between alloxan-diabetic and control dogs in the fractional conversion of plasma FFA to plasma TG. Reaven and Reaven[12] demonstrated that the synthesis and secretion of VLDL-TG were increased 24 h after the injection of streptozotocin (SZ) in rats, while chronic deficiency of insulin (seven days after SZ injection) was characterized by a decrease in VLDL released from liver.

The variability in TG synthesis in these studies may have been due to differences in animal species, differences in mode of induction of diabetes, and differences in the severity and duration of the diabetic state. In addition, the rate of output of TG by the liver of diabetic animals may not reflect the rate of esterification of FFA; thus, injection of anti-insulin serum into the rat and subsequent perfusion

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of the liver in vitro with FFA resulted in an initial phase of unchanged or increased secretion of TG followed by a phase of depressed release, while the hepatic TG content was unchanged.[13,14] Furthermore, because of the reported inhibition of apoprotein synthesis in liver of diabetic animals,[15,16] which could result in decreased VLDL secretion, it is difficult to assess TG synthesis based on release as VLDL. Importantly, these studies do not provide insights into the catalytic properties of the enzymes responsible for TG synthesis.

Therefore, we investigated (1) whether the accumulation of hepatic TG was related to the severity of diabetes, using SZ to induce diabetes, in the rat and (2) whether an increased synthesis of TG was a possible factor in its hepatic accumulation in diabetes. We also focused on the biochemical mechanisms responsible for the increased hepatic TG synthesis in diabetes.

EXPERIMENTAL PROCEDURES

MATERIALS

Phosphatidate, monolein, dilein, and triolein were obtained from Serdary Research Laboratories, London, Ontario, Canada; [U^{14}C]-sn-glycero 3-phosphate was obtained from New England Nuclear, Boston (specific activity, 117.4 mCi/mmol). The sources of other chemicals and insulin were as indicated earlier.[17] Fatty acid–free bovine serum albumin was obtained from Pentex, Kankakee, IL, and was further purified, as described,[18] to remove contaminants.[5,19]

METHODS

Induction of diabetes and determination of liver TG content. Male, Sprague-Dawley rats weighing 210 to 240 g were maintained ad libitum on Wayne chow (24.0% protein, 4.5% fiber, 4.0% fat, and the rest, carbohydrates) and tap water until killed. They were injected with either 60 mg SZ/kg body weight or 110 mg SZ/kg or an equal volume of buffer (control rats), as described previously.[17] Rats given 60 mg SZ/kg developed glucosuria but not ketonuria, while the rats injected with the higher dose of SZ had both glucosuria and ketonuria. No rat received insulin. The techniques for quick-freezing of liver samples, extraction of TG in chloroform:methanol (2:1 v/v), and determination of TG were as described.[17] Data are expressed as micromoles TG per gram lipid-free dry weight of liver. Plasma glucose, β-hydroxybutyrate (βHB), TG, and FFA were determined as described earlier.[17]

Biosynthesis of TG in liver homogenates. Since a consistent accumulation of TG was observed only in the ketotic diabetic rat (110 mg SZ/kg), we studied TG synthesis in this group. The rats exhibiting ketosis were maintained on insulin for five days.[17] The insulin treatment corrected glucosuria and ketonuria, and the rats gained weight at a rate comparable to that of controls. At the end of five days in some rats, saline injection replaced insulin to allow the return of the ketotic diabetic state, as described previously.[17] The insulin-treated rats received their last dose of the hormone four hours before being killed. Thus, TG synthesis was studied in three groups of rats: (1) buffer-injected controls, (2) ketotic diabetic rats (off insulin treatment), and (3) diabetic rats that continued to receive insulin until killed. The body weights of rats (20 in each group) when killed were as follows: control (258.2 ± 2.4 g, mean ± SE), ketotic diabetic (220.7 ± 2.9), and insulin treated (242.8 ± 3.4). Rats were killed, livers were removed and washed, and nuclear-free homogenates were prepared as described previously.[20] Homogenates were incubated under linear conditions with [U^{14}C]-sn-glycero 3-phosphate and cofactors. The incubation mixture contained 100 mM tris(HCl) buffer, pH 7.4, 12 mM DL-sn-glycero 3-phosphate (2 μCi), 200 μM CoA, 400 μM palmitate bound to purified bovine serum albumin, 5 mM ATP, and 2.5 mM Mg^{2+} (as chloride) and liver homogenates (about 800 μg protein): total volume was 500 μl. Incubations were done at 37° for 20 min. In the initial experiments, the concentrations of sn-glycero 3-phosphate, palmitate, and cofactors (ATP, CoA, and Mg^{2+}) were adjusted to ensure saturation and to establish that TG formation was linearly related to liver homogenate protein (0 to 1.6 mg) and to time of incubation (0 to 30 min).

The incubation mixture was mixed with reagents at 2° in the following order: 2.5 ml of chloroform:methanol mixture (1:2 v/v) containing 5 mM DL-glycero 3-phosphate, 0.8 ml of 0.2 N HCl, and 0.7 ml of chloroform. The suspension was centrifuged at room temperature for 20 min at 1000 × g. The top layer was discarded, and the bottom layer was washed twice using 2.5 ml of saline (pH 3.0) each time. The chloroform-rich layer was used for lipid analysis. In separate experiments, this extraction procedure was found to separate quantitatively the lipid and nonlipid compounds of the incubation mixture. The resolution of glycerolipids, counting of gel scrapings, and correction for quenching were done as described previously.[20] Phosphatidate and lysophosphatidate were separated on thin layer plates as described by Roncari and Murthy[20] and were identified as outlined by Jamdar and Fallon.[21]

Preparation of membrane-bound phosphatidate. Liver microsomal membrane-bound phosphatidate was prepared as described by Jamdar and Fallon[22] with slight modifications: normal rat liver microsomes were washed with 0.25 M sucrose, pH 7.4, and were suspended in fresh sucrose solution. The incubation mixture contained 50 mM tris (HCl) buffer of pH 7.4, 70 μM CoA, 3.5 mM ATP, 3.5 mM Mg^{2+}, 3.25 mM [U^{14}C]-sn-glycero 3-phosphate (2.0 μCi), 1.37 mM palmitate bound to purified bovine serum albumin, 50 mM NaF, and microsomes (about 800 μg protein) in a total volume of 700 μl. At the end of 60 min incubation at 37°C, the incubation flask was chilled in ice and 10 ml of ice-cold 50 mM tris (HCl) buffer, pH 7.4, was added. The microsomes were sedimented by centrifugation at 6 × 10^6 g min and were washed with cold 0.25 M sucrose, pH 7.4. The microsomes were suspended in fresh sucrose solution and were heated for five minutes at 100°C to inactivate the endogenous phosphatidate phosphohydrolase activity.

Phosphatidate phosphohydrolase (EC 3.1.3.4) activity. Phosphatidate phosphohydrolase activity of the liver-soluble fraction (6 × 10^6 g min supernate) was measured by incubating it with membrane-bound phosphatidate and determining the formation of diglyceride. The incubation mixture contained 100 mM tris (HCl) buffer of pH 7.0, 1 mM Mg^{2+}, 1.0 mg purified bovine serum albumin, varying concentrations of membrane-bound phosphatidate (Figure 3), and soluble fraction (about 1.2 mg protein) in a total volume of 500 μl. Incubation was for 10 min at 37°C. At the end

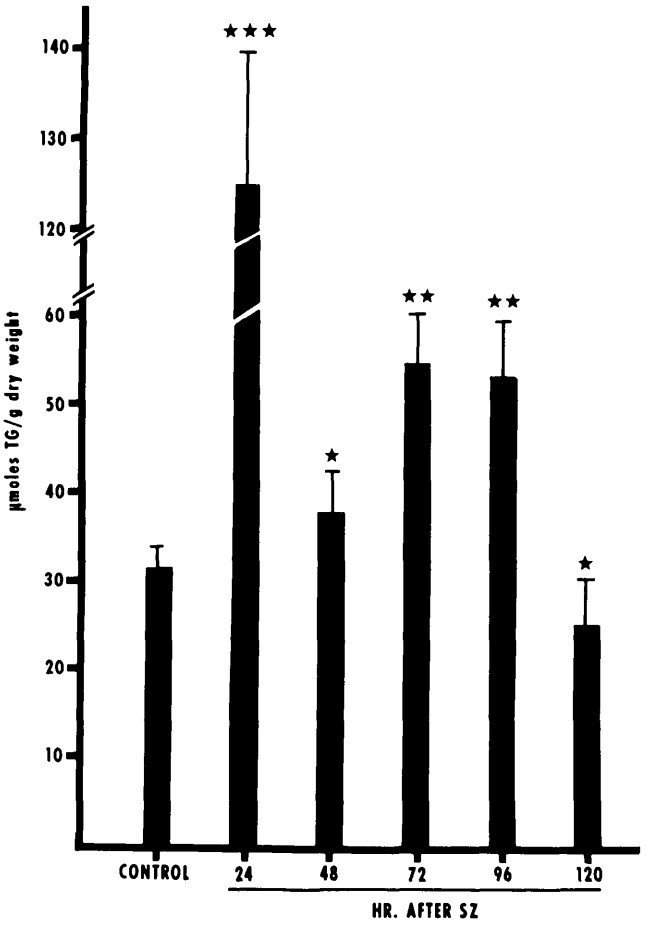

FIGURE 1. Hepatic triglyceride content (mean ± SE) in relation to time in nonketotic diabetic rats (seven rats in each group). Significance compared with control: *P > 0.05; **P < 0.02; ***P < 0.001.

of the experiment, the lipids were extracted and separated as described. The velocity of the reaction was linear with enzymic protein concentration (0 to 2.5 mg) and time of incubation (0 to 30 min). The kinetic data were analyzed,[23] and data on V_{max} were obtained using a Hewlett-Packard (model 9821A) computer equipped with a plotter. The correlation coefficients were 0.95 or better.

Diglyceride acyltransferase (EC 2.3.1.20) activity. The hepatic microsomal diglyceride acyltransferase activity of control, ketotic diabetic, and insulin-treated rats was determined according to the procedure described by Young and Lynen.[24] A linear relation between the microsomal protein concentrations (0 to 900 μg), the time of incubation (0 to 20 min), and the rate of TG formation was observed. Incubation was done for 10 min at 37°C employing about 400 μg microsomal protein.

RESULTS

The objective of the first series of experiments was to determine whether hepatic accumulation of TG has any relation to the severity of diabetes. Two different doses of SZ were injected to produce either nonketotic or ketotic diabetes. No rat received insulin. The diabetic state of these rats was characterized by analysis of plasma glucose, FFA, βHB, and TG. Our previous work demonstrated that nonketotic rats (60 mg SZ/kg) exhibited increased plasma concentrations of glucose, FFA, βHB, and TG.[17] Hepatic TG content in relation to time after SZ administration in these rats increased about threefold after 24 h; this accumulation of hepatic TG was not sustained, as evidenced by the decrease of TG content to control values at 120 h (Figure 1).

As noted previously,[17] overtly ketotic diabetic rats (110 mg SZ/kg) had plasma concentrations of FFA, βHB, and TG that were higher than in control rats. Hepatic TG content of these ketotic rats increased about 10-fold at 24 and 36 h and about 50-fold at 48 h after SZ administration (Figure 2).

The effects of insulin withdrawal and continued insulin treatment on the concentrations of plasma metabolites, liver TG content, and hepatic TG synthesis in vitro were studied next. Insulin treatment of the ketotic diabetic rats corrected the glucosuria and ketonuria; on insulin withdrawal for 28 h, they reverted to the ketotic diabetic state. This time period of insulin withdrawal was chosen since the rats exhibited glucosuria and ketonuria and their body weight was marginally (about 15%) less than that of buffer-injected controls. The plasma concentrations of glucose, FFA, βHB, and TG, as well as liver TG content of these ketotic diabetic rats were higher than those of the nondiabetic controls (Table 1). The hepatic TG content of the ketotic rats taken off insulin treatment was about threefold that of the control value (Table 1), while the hepatic TG content of the ketotic rats untreated with insulin for 24 h was about 10-fold the control value (Figure 2). The differences in plasma concentrations of metabolites (and other factors) between the two groups of rats may explain the differences in the hepatic TG content of these two groups of ketotic rats (Table 1 and reference 17).

Continued insulin treatment of the diabetic rat until sacrifice restored plasma concentrations of glucose, FFA, βHB, and TG to control values; however, the liver TG accumulation was only partially reversed (Table 1).

To determine whether increased synthesis of TG was a possible contributory factor in the hepatic accumulation of TG observed in the ketotic state, triglyceride synthesis in liver homogenates of control, ketotic diabetic, and insulin-treated rats was studied. In liver, a major pathway for TG synthesis is, first, the successive acylation of sn-glycero

FIGURE 2. Hepatic triglyceride content (mean ± SE) in relation to time in ketotic diabetic rats (eight rats in each group). Values at 24, 36, and 48 h are higher (P < 0.001) than those of control.

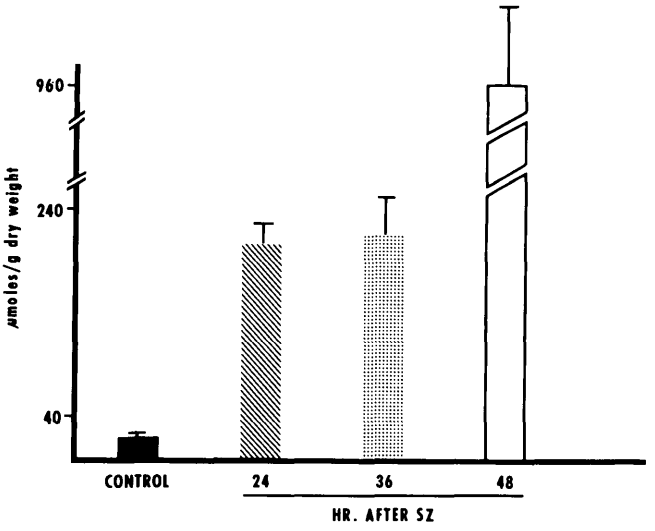

TABLE 1
Effects of ketotic diabetes and insulin treatment on plasma concentrations of various metabolites and liver triglyceride content

Metabolite	Control (C)	Ketotic diabetic (K)	Insulin treated (I)
Plasma			
glucose (mg/dl)	147.6 ± 2.3 (7)	601.5 ± 29.2 (8)*	153.4 ± 2.7 (7)‡,**
FFA (μEq/L)	335.3 ± 14.3 (8)	1489.6 ± 53.0 (8)*	286.1 ± 18.1 (7)‡,‖
βHB (μmol/dl)	7.3 ± 2.3 (8)	462.8 ± 123.5 (8)†	6.8 ± 2.7 (8)§,**
TG (μmol/dl)	69.7 ± 7.1 (8)	470.6 ± 31.5 (8)*	57.9 ± 7.2 (6)‡,**
Liver TG (μmol/g dry weight)	20.9 ± 1.9 (6)	65.9 ± 5.8 (6)*	29.4 ± 3.0 (6)‡,‖

The values are given as mean ± SE. The numbers in parentheses represent the number of rats in each group. The ketotic diabetic rats received no insulin for 28 h, whereas insulin-treated rats received it until they were killed.
Significance: *$P < 0.001$, †$P < 0.01$, both K vs. C; ‡$P < 0.001$, §$P < 0.01$, both I vs. K; ‖$P < 0.05$, **$P > 0.1$, both I vs. C.

3-phosphate to phosphatidate, followed by the hydrolysis of phosphatidate to diglyceride (DG), and, finally, the acylation of DG to form TG.[25]

In liver homogenates of ketotic diabetic rats, the recovery of radioactivity in DG and TG was increased while the recovery in phosphatidate was unchanged (Table 2). In agreement with earlier reports,[20] no radioactivity was recovered in the lysophosphatidate fraction of the incubation mixture, although the lipid extraction procedure used in the present experiments would have extracted this lyso compound.[26] Insulin treatment of the diabetic rats produced a partial, but significant, reversal of the increased recovery of radioactivity in DG observed in ketotic diabetes; however, insulin treatment did not reverse the increased recovery of label in TG (Table 2).

The phosphatidate phosphohydrolase activity of liver-soluble fraction of control, ketotic diabetic, and insulin-treated rats was studied directly, employing membrane-bound phosphatidate as the substrate. The results demonstrate that the V_{max} of this enzyme increased in liver-soluble fractions of ketotic rats, but the phosphatidate phosphohydrolase activity reverted to control values on treatment of the ketotic diabetic rats with insulin (Figure 3 and Table 3).

Diglyceride acyltransferase catalyzes the transfer of a long-chain acyl group to DG to form TG and is found almost exclusively in the microsomes in liver.[25] The activity of this enzyme was measured in the hepatic microsomes of control, ketotic diabetic, and insulin-treated rats. The results demonstrate that the activity increased in ketotic diabetes but reverted to control values with insulin treatment of the diabetic rats (Table 3).

DISCUSSION

It is known that the rate of hepatic TG synthesis is determined by the plasma or perfusate concentration of FFA and by the hormonal and nutritional state of the animal.[27] McGarry and Foster[28] studied the uptake and fate of ^{14}C-oleic acid in the perfused livers of fed and fasted rats; though the hepatic uptake of oleic acid was unaffected by the nutritional state, esterification of oleic acid to TG was depressed in livers of fasted rats. Increased conversion of oleic acid to TG in perfused livers of rats given estrogens[27] and in perfused livers of rats refed after fasting[29] was observed, although uptake of fatty acid by the liver was unaffected by the altered nutritional or hormonal state of the rat. Thus, it appears that the metabolic state of the liver is a major determinant of the rate of hepatic TG synthesis.

The results of this study are that a significant and consistent accumulation of hepatic TG was observed only in the severely ketotic diabetic state. These observations suggest a close relationship between increased ketone body concentration and TG accumulation in liver. Observations made in other laboratories support such a relationship. Thus, Meier et al.[30] observed that ketosis preceded increased TG formation in the liver of alloxan-diabetic rats. In isolated perfused livers of rats treated with anti-insulin serum, Woodside and Heimberg[3] observed that the rate of hepatic ketogenesis was maximal before hepatic TG content increased significantly. In the perfused normal rat heart, Olson[31] found that the addition of acetoacetate to the perfusate containing palmitate resulted in increased conversion of palmitate to TG. Further studies are required to document and define this apparent relationship between ketosis and TG accumulation in liver.

The hepatic accumulation of TG could have been due to an increased synthesis of TG or its decreased release from liver or both. Heimberg and colleagues[13,14] demonstrated that TG synthesis in livers of normal and diabetic rats was related to the perfusate FFA concentration, its uptake and esterification. These studies emphasized the role of substrate concentration as a regulator of hepatic TG synthesis. Cell-free preparations of liver were employed by other investigators to study the biochemical changes that could explain the accumulation of hepatic TG, e.g. Fallon and co-workers.[32,33] The present study was designed to determine whether increased synthesis was present in liver of ketotic diabetic rats at a time when there was hepatic ac-

TABLE 2
Effects of diabetes and insulin treatment on radioactive glycerolipid synthesis

		Diabetes	
Glycerolipid	Control (C)	Ketotic (K)	Insulin treated (I)
Phosphatidate	40.80 ± 1.34	38.40 ± 5.04	43.35 ± 3.24
Diglyceride	11.35 ± 1.43	25.90 ± 1.71	18.75 ± 1.56
Triglyceride	3.85 ± 0.60	11.80 ± 0.91	8.95 ± 0.84

Values, expressed as nanomoles per milligram protein per 20 min of incubation, represent mean ± SE. Each number was obtained from four different experiments. In each experiment, livers from four rats in each group were pooled and homogenized.
Significance: Phosphatidate—C vs. K, $P > 0.1$; K vs. I, $P > 0.1$; I vs. C, $P > 0.1$. Diglyceride—C vs. K, $P < 0.001$; K vs. I, $P < 0.05$; I vs. C, $P < 0.02$. Triglyceride—C vs. K, $P < 0.001$; K vs. I, $P > 0.05$; I vs. C, $P < 0.005$.

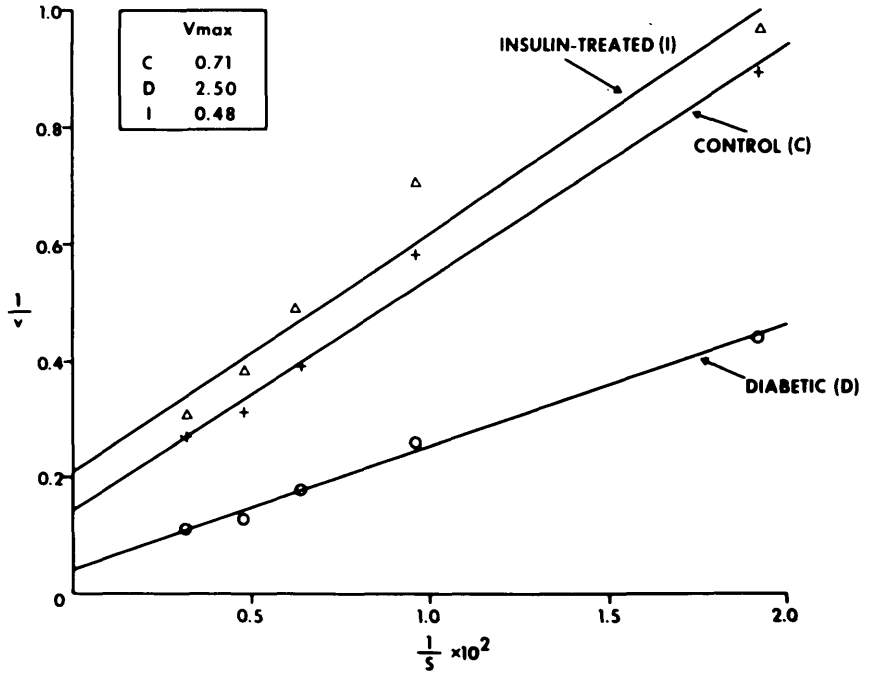

FIGURE 3. Kinetic analysis of the liver-soluble phosphatidate phosphohydrolase. Incubation conditions are described in the text. V_{max} (nmol DG/mg protein/min) is shown in inset.

cumulation of TG. TG synthesis was studied using liver homogenates of control, ketotic diabetic, and insulin-treated rats.

The results of our study on TG synthesis demonstrate an increased recovery of radioactivity in the neutral lipid (DG, TG) fractions in diabetes, while the recovery of radioactivity in phosphatidate is unaffected by the diabetic state. That this increased recovery of radioactivity is due to increased synthesis and not to possible changes in hepatic pool size of sn-glycero 3-phosphate in diabetes is indicated by the following considerations: sn-glycero 3-phosphate concentration in liver of ad libitum–fed normal rats is in the range of 158 to 940 nmol/g wet weight of liver.[34–37] Hepatic phosphatidate concentration ranges from 0 to 390 nmol/g wet weight of liver.[25,38,39] Assuming the highest values, namely 940 nmol for sn-glycero 3-phosphate and 390 nmol for phosphatidate, and assuming that both these metabolites were recovered in the liver supernates of 800 *g* employed for incubation, this would have represented the presence of about 3.3 nmol of sn-glycero 3-phosphate and about 1.3 nmol of phosphatidate in the liver homogenate added to each incubation tube (equivalent to 3.5 mg of liver tissue), while 3000 nmol of radioactive glycero 3-phosphate was added to each incubation tube to ensure saturation. Thus, any changes in the pool size of sn-glycero 3-phosphate in the liver of ketotic rats did not influence the recovery of radioactivity in the neutral lipids. In fact, the hepatic glycerophosphate concentration is unaffected by the diabetic state.[40]

The amount of phosphatidate present in the liver homogenate added to incubation mixtures represents, at the most, about 3% of the amount synthesized. Any change in the pool size of phosphatidate in diabetes is unlikely to influence neutral lipid synthesis. Furthermore, phosphatidate synthesis by liver homogenates was unaffected by the ketotic diabetic state (Table 2).

TG synthesis was studied by incubating liver homogenates with saturating concentrations of palmitate, sn-glycero 3-phosphate, Mg^{2+}, ATP, and CoA. The increased synthesis of neutral lipids in liver homogenates of diabetic rats (Table 2) suggested that activities of the enzymes responsible for neutral lipid synthesis were increased. Despite treatment of the diabetic rats with insulin for five days in dosages that reversed the increased concentrations of plasma metabolites and promoted weight gain of rats, augmented TG synthesis was not corrected, while the conversion of phosphatidate to DG was partially normalized (Table 2).

To study the biochemical mechanisms responsible for the increased DG and TG synthesis in ketosis, the activity of the two key enzymes responsible for DG and TG synthesis, namely phosphatidate phosphohydrolase and di-

TABLE 3
Specific activity of hepatic soluble phosphatidate phosphohydrolase and microsomal diglyceride acyltransferase

Enzyme	Specific activity (nmol/mg protein/min)		
	Control (C)	Ketotic diabetic (K)	Insulin treated (I)
Phosphatidate phosphohydrolase	0.77 ± 0.13 (3)	2.14 ± 0.19 (3)	0.76 ± 0.21 (3)
Diglyceride acyltransferase	3.40 ± 0.68 (3)	4.86 ± 1.41 (3)	2.76 ± 0.89 (3)

Values represent mean ± SE. Number in parentheses represents number of experiments. In each experiment, livers from three rats in each group were pooled and homogenized.

Significance: Phosphatidate phosphohydrolase—C vs. K, $P < 0.01$; K vs. I, $P < 0.01$; C vs. I, $P > 0.1$. Diglyceride acyltransferase (paired analysis)—C vs. K, $P < 0.05$; K vs. I, $P < 0.05$; C vs. I, $P > 0.1$.

glyceride acyltransferase, respectively, were measured. Phosphatidate phosphohydrolase, distributed in the soluble fraction and in microsomes,[25] possesses characteristics that indicate the existence of different enzymes in these two subcellular fractions.[22,25,41] Furthermore, since the activity of this enzyme is rate-limiting in hepatic TG synthesis in vitro,[25,42] an increase in the activity of this enzyme could result in increased conversion of phosphatidate to DG, thereby increasing the total rate of TG synthesis in vitro. A regulatory role was suggested for phosphatidate phosphohydrolase in TG synthesis in vitro[25] and in vivo,[32,43] since the activity of this enzyme is increased in conditions of increased TG synthesis, such as carbohydrate feeding,[32] genetic obesity,[44] after subtotal hepatectomy,[45] and after ethanol administration.[46] Phosphatidate phosphohydrolase of the soluble fraction was suggested to play an important role in TG synthesis.[25]

In the present experiments, the activity of soluble phosphatidate phosphohydrolase was assessed directly using membrane-bound phosphatidate as the substrate. The activity (V_{max}) of the hepatic soluble enzyme was increased in ketotic diabetes and reverted to control values on treatment of the ketotic rats with insulin. We realize that the V_{max} measurement of an enzyme reflects the capacity of the enzyme for catalytic activity and the results may not reflect the actual rate of conversion of metabolites in the cell. The data do not indicate whether the increased catalytic activity of phosphatidate phosphohydrolase was due to modification of the kinetic properties of the normal enzyme by some factor(s) associated with ketotic diabetes or to a new enzyme.

Diglyceride acyltransferase catalyzes the conversion of DG to TG and is considered a key enzyme in the glycerophosphate pathway. DG, like phosphatidate, serves as a common intermediate for both triglyceride and phospholipid biosynthesis,[47] and, thus, the activity of phosphatidate phosphohydrolase and diglyceride acyltransferase could determine the flow of substrates into the phospholipid or triglyceride biosynthetic pathways. The activity of hepatic microsomal diglyceride acyltransferase was increased in ketotic diabetes (Table 3), in agreement with observations of Young and Lynen.[24] The results of the present study showed that insulin treatment of diabetic rats reversed the activity of this enzyme to control values.

Observations in this study demonstrated a correlation between the increased hepatic TG content in ketosis, the rate of TG synthesis in vitro in liver homogenates of ketotic rats, and the increased activity of the key enzymes of neutral lipid synthesis in ketosis. The hepatic TG content of insulin-treated rats, although greatly reduced from values seen in ketosis, remained higher than that of control rats and correlated with augmented TG synthesis in vitro in the insulin-treated rats (Table 2). The continued high rates of TG synthesis in liver homogenates of the insulin-treated rats were surprising and were in contrast to normal phosphatidate phosphohydrolase and diglyceride acyltransferase activity after insulin treatment (Table 3). The reasons for the continued high rates of triglyceride synthesis in insulin-treated rats are not clear. The control of hepatic triglyceride synthesis may be more complex than what the results of this study suggest; for example, Roncari and Mack[48] purified two cytosolic factors from normal rat liver and adipose tissue that promoted triglyceride synthesis when added to microsomes—at least one factor appeared to be a nonenzymic activator. It is possible that the activity or the content of these or similar factors could play a role in increased triglyceride synthesis in diabetes.

Finally, the correlation between hepatic TG content and TG synthesis in control, ketotic, and insulin-treated rats reported here as well as the modulation of the activities of the key enzymes of neutral lipid synthesis in states of insulin deprivation and insulin replenishment suggest a physiologic role of these changes in the hepatic accumulation of TG in ketotic diabetes.

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