Studies on the mechanism of hypertriglyceridemia in the genetically obese Zucker rat[1]

Chi-Sun Wang, Nobuhiro Fukuda,[2] and Joseph A. Ontko

Laboratory of Lipid and Lipoprotein Studies, and Cardiovascular Research Laboratory, Oklahoma Medical Research Foundation, and Department of Biochemistry and Molecular Biology, College of Medicine, University of Oklahoma, Oklahoma City, OK 73104

Abstract The possibility that impaired removal of lipoprotein triglyceride from the circulation may be a participating factor in the hypertriglyceridemia of the obese Zucker rat was examined. We found no significant differences in the heparin-released lipoprotein lipase (LPL) activities of the adipose tissue, skeletal muscle, and heart (expressed per gram of tissue) from the lean and obese Zucker rats. Furthermore, the kinetic properties of adipose tissue and heart LPL from the lean and obese rats were similar, indicating that the catalytic efficiency of the enzyme was unaltered in the obese animals. The postheparin plasma LPL activities of lean and obese rats were also similar. However, the postheparin plasma hepatic triglyceride lipase (H-TGL) activity in the obese rats was elevated. The higher activity of H-TGL could not alleviate the hypertriglyceridemia in these animals. Since hypertriglyceridemia in the obese rats could also be due to the hepatic production of triglyceride-rich lipoproteins which are resistant to lipolysis, we therefore isolated very low density lipoproteins (VLDL) from lean and obese rat liver perfusates and examined their degradation by highly purified human milk LPL. Although certain differences were observed in hepatic VLDL triglyceride fatty acid composition, the kinetic patterns of LPL-catalyzed triglyceride disappearance from lean and obese rat liver perfusate VLDL were similar. The isolated liver perfusate VLDL contained sufficient apolipoprotein C-II for maximum lipolysis. These results indicate that impaired lipolysis is not a contributing factor in the genesis of hypertriglyceridemia in the genetically obese Zucker rat. The hyperlipemic state may be attributed to hypersecretion of hepatic VLDL and consequent saturation of the lipolytic removal of triglyceride-rich lipoproteins from the circulation.—**Wang, C-S., N. Fukuda, and J. A. Ontko.** Studies on the mechanism of hypertriglyceridemia in the genetically obese Zucker rat. *J. Lipid Res.* 1984. **25:** 571–579.

Supplementary key words fatty acid composition • hepatic VLDL • hepatic triglyceride lipase • kinetics • lipoprotein lipase • perfused liver • polyunsaturated fatty acids • saturated fatty acids • triglyceride • VLDL

The genetically obese Zucker rat (2, 3) is hyperlipemic, as characterized by markedly elevated levels of triglyceride and moderately increased concentrations of cholesterol in the blood. Both very low density lipoproteins (4) and chylomicron remnant particles (5) accumulate in the circulation of these animals. Previous studies have implicated increased hepatic production of very low density lipoproteins (VLDL) in the development of this hypertriglyceridemic state (6, 7).

In addition to overproduction of triglyceride-rich lipoproteins by the liver, a decreased lipolytic system in the circulation may be a contributing factor, since lipolysis represents a rate-limiting step in the uptake of triglyceride fatty acids by extrahepatic tissues (8–11). Others have found a normal or elevated activity of total lipoprotein lipase (LPL) in muscle and adipose tissue in the genetically obese Zucker rat (12, 13). Since defective lipolysis could nevertheless result from a low level of functional LPL in the luminal surface of the capillary endothelium, from depressed hepatic triglyceride lipase (H-TGL), or from the hepatic production of VLDL which are resistant to lipolysis, we have examined these possibilities. The results indicate that overproduction of triglyceride-rich lipoproteins by the liver is primarily responsible for the hyperlipemia in this genetically obese syndrome.

MATERIALS AND METHODS

Animals

Male lean and obese Zucker rats were obtained from Dr. Ruth Young at the University of Massachusetts. They were housed individually (room temperature, 22°C) and allowed free access to Purina laboratory chow and water. Lighting was controlled (6 AM–6 PM, light; 6 PM–6 AM, dark). Between 9:00–9:30 AM, rats were anesthetized (7). The rats were 14–18 weeks of age. Blood was obtained by heart puncture just before inserting a cannula into the portal vein. Livers were removed for perfusion by

Abbreviations: VLDL, very low density lipoproteins; LPL, lipoprotein lipase; H-TGL, hepatic triglyceride lipase; SDS, sodium dodecyl sulfate.

[1] This work has been presented in abstract form (1).

[2] Present address: Laboratory of Biochemistry and Nutrition, Department of Agricultural Chemistry, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan.

recirculation (7). Blood was allowed to clot for 5 hr at 5°C. Serum was separated by centrifugation at 3,000 rpm for 30 min at 5°C.

Extraction of tissue LPL

Tissues (adipose tissue, heart, and skeletal muscle) were removed from anesthetized rats (within 1 hr of Nembutal administration) and placed immediately in cold 0.15 M NaCl. Perirenal adipose tissue, the entire heart, and the entire right upper and lower leg skeletal muscles were obtained for analysis. The tissues were rinsed three or four times with cold 0.15 M NaCl; they were then blotted with filter paper and placed in small sterile specimen containers and frozen within 5 min after removal. All tissues were kept frozen until used (within 1 week). The LPL activity was stable during this period of storage. The LPL in the heparin extract was unstable, however. Therefore, extraction of LPL was performed on the day of assay.

On the day of analysis the frozen tissue was weighed, cut into 3–4-mm pieces, and extracted with Krebs-Ringer phosphate buffer (0.5 g of tissue/ml; at pH 7.4) containing 1 mg/ml of heparin. The tissues (approximately 1 g) were incubated in a shaking water bath at 25°C for 30 min. The incubation mixture was removed from the tissue and centrifuged at 2000 rpm at 4°C for 15 min. The supernatant was removed and kept in an ice bath until LPL activity assay (within 10 min). Under these experimental conditions no lipase activity was observed in the heparin extracts without added serum, indicating that storage by freezing and subsequent thawing did not liberate significant intracellular lysosomal or hormone-sensitive lipase.

Measurement of LPL activity from tissues

The assay of LPL activity was performed in a final assay mixture (200 μl) in 50 mM NH_4OH-HCl buffer, pH 8.5, containing 60 mg/ml bovine serum albumin, 25 mM $(NH_4)_2SO_4$, 10 mM trioleoylglycerol (0.1 μCi/μmol tri [1-^{14}C]oleoylglycerol), 2.5 mg/ml of Triton X-100, 20 μl of rat serum as activator, and 80 μl of the tissue extracts. The rat serum utilized as activator contained no detectable endogenous lipase activity. Triton X-100 was employed for emulsification (14), rather than phospholipid, since phospholipid may also be a substrate for LPL (15). The incubation was performed at 37°C in a shaking water bath for 1 hr. The reaction was terminated by adding 3.2 ml of chloroform–heptane–methanol 5:4:5.6 (v/v/v) and 1 ml of 0.2 N NaOH. After centrifugation 1.2 ml of the top layer was mixed with 10 ml of Instagel and radioactivity was measured in a Packard liquid scintillation counter. One unit of lipase activity is defined as 1 μmol of fatty acid released per hr at 37°C.

Measurement of postheparin plasma lipolytic activities

Postheparin plasma was obtained from lean and obese Zucker rats 2 min after intravenous injection of 1000 IU heparin/kg body weight (16). The plasma was separated by centrifugation at 4°C and stored at −20°C. The determinations of LPL and H-TGL were performed as described previously using the micro-heparin-Sepharose column procedure (17). One ml of postheparin plasma was mixed with 3 ml of 50 mM NH_4OH-HCl buffer, pH 8.5, and applied to a micro-heparin-Sepharose column (7 mm diameter, packed with heparin-Sepharose to give a 2-cm column). After the sample was loaded, the column was washed with 4 ml of 0.3 M NaCl containing 50 mM NH_4OH-HCl buffer; then LPL and H-TGL were eluted together with 4 ml of buffer containing 10 mg/ml of heparin. H-TGL was measured directly from the heparin-eluted fraction in the absence of the activator (human serum) and the total activity was measured in the presence of activator. The difference between total activity and H-TGL activity represents the LPL activity (17). Because it has been reported that the activation of LPL by rat serum varied greatly between different sera and was optimum within a relatively narrow range, we used human serum as activator (16).

Isolation of rat liver perfusate VLDL

The liver perfusion equipment and procedures were as employed and described previously (7). The bovine serum albumin in the perfusion medium (fraction V fatty acid-free, Miles Laboratories) was devoid of apolipoprotein C-II by LPL activity assay. VLDL was isolated from 30 ml of cell-free perfusate at the end of the perfusion period by ultracentrifugation after layering with 10 ml of NaCl (d 1.006 g/ml) containing 0.2% EDTA (7).

Purification of human milk LPL

LPL was prepared from the acetone-diethyl ether powder of human milk cream and purified by heparin-Sepharose affinity chromatography (15). The powder (40 mg) was dispersed with a pestle and solubilized by stirring for 10 min at room temperature in 4 ml of 50 mM HCl-NH_4OH buffer, pH 8.5, containing 0.1% Triton X-100. The mixture was centrifuged at low speed for 2 min. The supernatant fraction was collected and applied to a small heparin-Sepharose column (0.7 × 2 cm). After loading the sample, the column was washed first with 4 ml of 50 mM NH_4OH-HCl buffer, pH 8.5, containing 0.3 M NaCl and then with 4 ml of the same buffer containing 0.72 M NaCl. Following these washes, the LPL was eluted with 3 ml of 50 mM HCl-NH_4 OH buffer containing heparin (10 mg/ml). The LPL activity in this heparin extract was 30–40 units/ml.

Lipolysis of rat liver perfusate VLDL

In a final volume of 6 ml, the lipolysis mixture contained liver perfusate VLDL (0.3 mg of triglyceride per ml), bovine serum albumin (60 mg/ml), 50 mM NH_4OH-HCl, pH 8.0, and human milk LPL (1 ml of heparin eluate). Rat serum and Triton X-100 were not included in these assays. At various time intervals, duplicate samples (0.5 ml) were removed and added to 4 ml of n-heptane–isopropanol 3:7 (v/v) containing 50 μg of cholesteryl butyrate as internal standard for gas–liquid chromatographic analysis of triglycerides as described below.

Gas–liquid chromatography

Aqueous samples (0.5 ml) in duplicate were transferred to tubes containing 4 ml of n-heptane–isopropanol 3:7 (v/v) containing 50 μg of cholesteryl butyrate as internal standard for neutral lipid analysis by gas–liquid chromatography. After being acidified with 2.5 ml of 0.033 N H_2SO_4, the mixture was vortexed for 30 sec; the organic phase containing the triglycerides and the internal standard was transferred to a 3-ml conical tube; and the solvent was evaporated under nitrogen. The residue was redissolved in 100 μl of n-hexane and 2-μl aliquots were injected into the gas chromatograph. The pattern of separation of triglyceride molecular species has been shown previously (15). The major molecular species are designated TG-50, TG-52, TG-54, and TG-56, according to the total carbon content of the acyl groups.

Other methods

Protein content was determined by a modification (18) of the procedure of Lowry et al. (19). Bovine serum albumin was used as the standard. Urea-SDS-polyacrylamide gel electrophoresis was carried out in 7% acrylamide gel in the presence of 0.1% sodium dodecyl sulfate and 8 M urea as previously described (20).

Analysis of results

The K_m values derived from Lineweaver-Burk plots were based on least squares linear regression analysis (21) assuming a constant variance of reaction velocity computed (with a Compucorp 344 statistician microcomputer). The measured initial rates had coefficients of variation of less than 10%. For statistical analyses the Student's *t*-test and Wilcoxon rank sum test were employed where indicated.

RESULTS

LPL activity in tissue extracts

The incubation of tissues with heparin resulted in the release of LPL into medium. Our initial experiment was to examine the concentration of heparin required for the optimum release of LPL. The study indicated that 1 mg/ml of heparin was sufficient for the maximal release of LPL from the various tissues examined. In the absence of heparin, approximately 5–10% of the maximal releasable LPL and protein were released into the medium. Because LPL was not the only protein released during incubation of tissues with heparin, we also measured the amounts of protein released from the tissues during incubation. On the average, 4, 6, and 14 mg of protein/g of tissue were released from adipose tissue, heart, and skeletal muscle, respectively. There was no apparent difference between lean and obese Zucker rats with respect to the amounts of protein released from each tissue. SDS polyacrylamide gel electrophoresis patterns of the heparin-releasable fraction of adipose tissue from lean and obese Zucker rats are shown in **Fig. 1.** There was no apparent difference between these patterns. A much greater heterogeneity of the heparin-releasable proteins from heart and skeletal muscle was observed, as compared with adipose tissue. Because of the complexity of these polyacrylamide gel patterns, we did not compare in detail

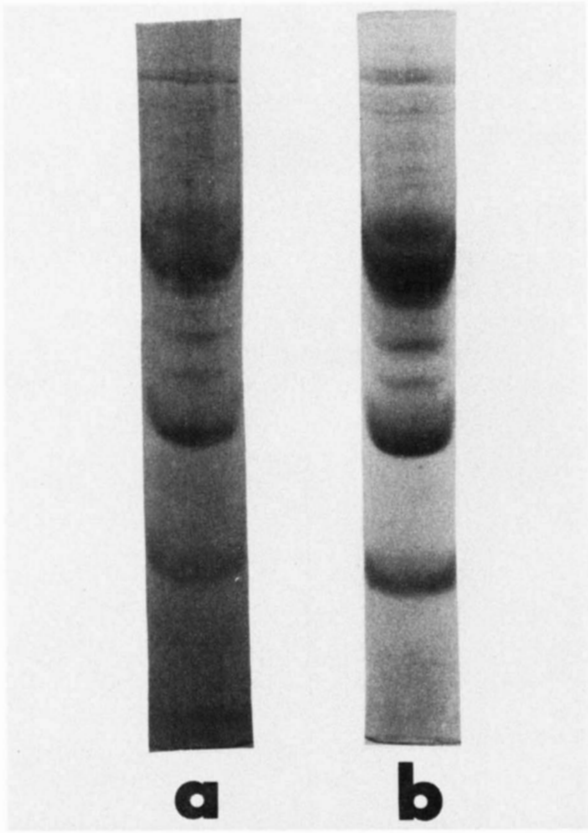

Fig. 1. SDS polyacrylamide gel electrophoresis patterns of heparin-releasable fractions of adipose tissue from lean (a) and obese (b) Zucker rats.

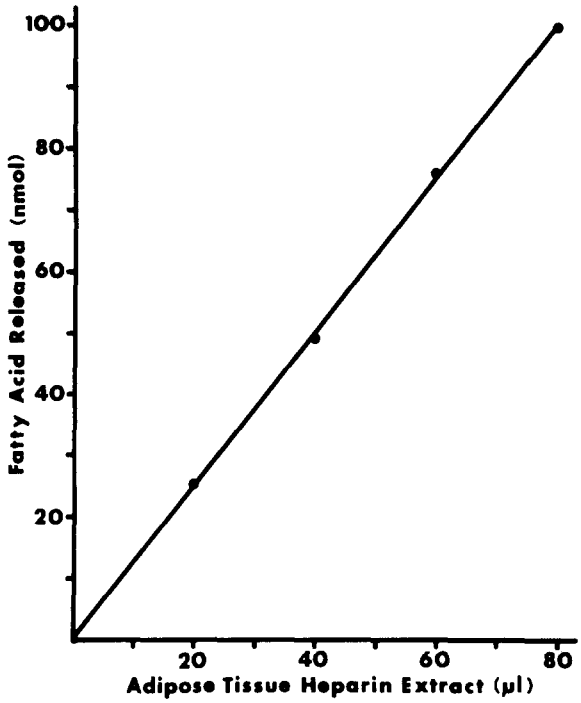

Fig. 2. The linear relationship between lipolytic activity and the amount of enzyme of heparin extract of adipose tissue from lean Zucker rat. A similar linear response was observed in the extract of obese Zucker rat adipose tissue.

the heparin extracts from skeletal muscle and heart of the lean and obese rats.

In our assay system, a linear relationship between the amount of enzyme and LPL activity was observed from adipose tissue (**Fig. 2**) as well as from heart and skeletal muscle tissue extracts. This is a prerequisite for utilizing the activity as a measure of enzyme concentration. The LPL activities in heparin extracts of adipose tissue, heart and skeletal muscle are shown in **Table 1.** We found no significant differences in LPL activities of these tissues between lean and obese rats. As expected, skeletal muscle had the lowest activity of the three tissues examined. The LPL activities found in heart and adipose tissue were similar.

TABLE 1. Tissue lipoprotein lipase activities of lean and obese Zucker rats

Tissue	Lean (6)[a]	Obese (6)	*P*
	µmol fatty acid release/g tissue per hr		
Adipose tissue	2.62 ± 0.28[b]	2.30 ± 0.23	NS[c]
Skeletal muscle	0.59 ± 0.08	0.62 ± 0.05	NS
Heart muscle	3.03 ± 0.36	2.11 ± 0.26	NS

[a] The numbers in parentheses indicate the number of animals in each group.
[b] Mean ± SEM.
[c] Not significant.

Kinetic properties of LPL

Because defective lipolysis may result from a structural alteration in LPL due to genetic mutation, we examined the kinetic properties of LPL from tissue extracts of lean and obese Zucker rats. Under identical assay conditions, the LPL from heparin extracts of adipose tissue from lean and obese rats showed a similar relationship between substrate concentration and enzyme activity, which followed Michaelis-Menten kinetics (**Fig. 3a**). The apparent K_m values for the adipose tissue enzyme derived from lean and obese rats were similar (0.95 and 1.00 mM, respectively). In contrast, the LPL from heparin extracts of heart tissue did not follow Michaelis-Menten kinetics (Fig. 3b); the reaction rate of heart LPL showed a sigmoidal response to the triolein concentration. There is no corresponding K_m for this type of reaction kinetics. Despite this, the LPL activities of the heparin extracts from both lean and obese rats showed similar kinetic patterns. Accordingly, no apparent kinetic alterations in

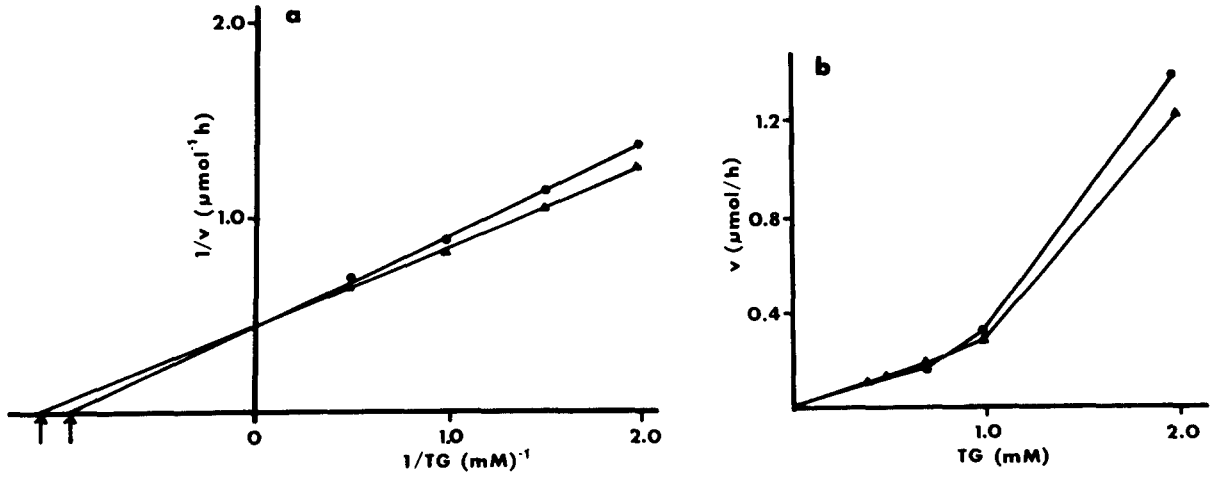

Fig. 3. Kinetic properties of LPL from (a) adipose tissue and (b) heart of lean (▲) and obese (●) Zucker rats.

TABLE 2. Postheparin plasma lipolytic activities of lean and obese Zucker rats

	Body Weight	Liver Weight	Postheparin Plasma	
			Lipoprotein Lipase	Hepatic Lipase
	g	*g*	*μmol fatty acid release/ml plasma per hr*	
Lean (5)[a]	314.0 ± 10.9[b]	11.1 ± 0.4	23.7 ± 2.0	26.9 ± 2.7
Obese (4)	438.8 ± 15.3	20.5 ± 0.8	22.6 ± 1.4	45.8 ± 2.5
P	<0.001	<0.001	NS	<0.005

[a] The numbers in parentheses indicate the number of animals in each group.
[b] Mean ± SEM.

the LPL activities from lean and obese rat adipose tissue and heart were detected. Because of the low LPL activity in heparin extract of skeletal muscle, we did not perform kinetic analyses of LPL from this tissue.

Lipolytic activities in postheparin plasma

For the measurement of LPL and H-TGL, we found that it was necessary to partially purify the postheparin lipolytic activities by heparin-Sepharose affinity chromatography to obtain reliable results, possibly because of interference by high concentrations of triglyceride-rich lipoproteins.

Consistent with the lack of significant difference between lean and obese rats in the LPL activity from adipose tissue, skeletal muscle, and heart (Table 1), we also found that there was no significant difference in postheparin plasma LPL activities (**Table 2**). However, we observed a significantly higher activity of H-TGL (1.7-fold) in obese rats.

Characterization of liver perfusate VLDL triglyceride and its rate of degradation by LPL

To examine the possibility that the obese Zucker rat liver produces VLDL which is resistant to lipolysis, hepatic VLDL was harvested from isolated rat liver perfusates and subjected to lipolytic degradation. The animals employed for these perfusion experiments are described in **Table 3.** The obese rats were hypertriglyceridemic and net triglyceride secretion by the perfused livers from these animals was elevated 5- to 6-fold.

Our previous studies using synthetic monoacid triglycerides as substrates for LPL indicated that the fatty acid composition of the triglyceride molecule may play a role in determining the rate of lipolysis (22). Therefore, we examined the triglyceride fatty acid composition of the liver perfusate VLDL. As shown in **Table 4,** specific differences in the fatty acid composition of the VLDL triglyceride secreted by lean and obese rat livers were observed. Among the major components of triglyceride fatty acids, namely palmitate, oleate, and linoleate, only the percent composition of oleate was similar. The palmitate content was increased and the relative percentage of linoleate was decreased. Significant differences in the percent composition of palmitoleic, stearic, and in the polyunsaturated fatty acids, 20:4, 20:5, and 22:6, were also observed.

The fatty acid composition of liver triglyceride showed a similar reciprocal alteration in palmitate and linoleate with a higher content of the former and a lower percentage of the latter in the obese rats (Table 4). Similar alterations in the percentages of 16:1, 20:4, 20:5, and 22:6, as compared with the composition of VLDL triglyceride, were also observed. When liver triglyceride fatty acid composition was compared with that of the

TABLE 3. Plasma, liver, and liver perfusate triglycerides in lean and obese Zucker rats

	Lean (4)[a]	Obese (4)	*P*
Body weight (g)	322 ± 6[b]	474 ± 12	<0.001
Liver weight (g)	12.2 ± 0.3	19.7 ± 0.6	<0.001
Plasma triglyceride (μmol/dl)	90.6 ± 4.7	668.2 ± 69.1	<0.001
Liver triglyceride[c] (μmol/g)	11.6 ± 0.4	53.6 ± 11.2	<0.01
Liver perfusate triglyceride[d] (μmol/liver/225 min)	23.1 ± 2.3	127.1 ± 25.0	<0.01

[a] The number of animals in each group is in parentheses.
[b] Standard error of the mean.
[c] Analyzed at the end of the 225-min perfusion.
[d] Total triglyceride accumulation in the perfusate after 225 min of recirculation.

TABLE 4. Triglyceride fatty acid composition of serum, liver, and liver perfusate of lean and obese Zucker rats

		Triglyceride								
		14:0	16:0	16:1	18:0	18:1	18:2	20:4	20:5	22:6	Unknown
Liver perfusate	Lean (4)[a]	1.1 ± 0.1[b]	17.2 ± 0.2	3.0 ± 0.4	2.8 ± 0.2	53.9 ± 0.3	9.9 ± 0.2	1.2 ± 0.1	0.5 ± 0.1	3.9 ± 0.2	5.6 ± 0.3
	Obese (4)	1.5 ± 0.2	25.6 ± 0.2	5.3 ± 0.4	1.9 ± 0.1	52.8 ± 1.8	5.1 ± 0.5	0.4 ± 0.1	0.7 ± 0.1	3.0 ± 0.4	3.7 ± 0.3
	P[c]	NS	<0.05	<0.05	<0.02	NS	<0.02	<0.02	<0.02	<0.05	<0.02
Liver	Lean (4)	2.0 ± 0.8	22.0 ± 0.5	3.0 ± 0.4	3.6 ± 0.5	40.0 ± 0.9	14.7 ± 0.6	1.2 ± 0.1	0.2 ± 0.1	5.5 ± 0.3	6.8 ± 0.5
	Obese (4)	1.6 ± 0.2	35.8 ± 1.0	6.6 ± 0.3	3.2 ± 0.3	38.3 ± 1.7	7.4 ± 0.8	0.5 ± 0.1	0.5 ± 0.1	2.1 ± 0.6	3.9 ± 0.7
	P	NS	<0.05	<0.05	NS	NS	<0.02	<0.02	<0.02	<0.02	<0.02
Serum	Lean (4)	2.5 ± 0.4	25.6 ± 0.5	3.6 ± 0.4	5.9 ± 0.8	25.1 ± 0.6	21.2 ± 0.6	1.2 ± 0.1	0.3 ± 0.1	5.9 ± 0.4	7.0 ± 0.9
	Obese (4)	1.9 ± 0.1	34.4 ± 0.7	7.2 ± 0.7	3.9 ± 0.1	30.7 ± 0.7	11.5 ± 0.5	0.7 ± 0.3	0.3 ± 0.1	3.3 ± 0.5	5.3 ± 0.7
	P	NS	<0.05	<0.05	<0.02	<0.05	<0.02	NS	<0.02	<0.02	NS

[a] The numbers in parentheses indicate the number of animals analyzed in each group.
[b] The values shown are the mean ± SEM.
[c] The Wilcoxon rank sum test, which corresponds to the Mann-Whitney U test, was employed. The lowest possible P value with the number of values statistically analyzed (four in each group) was <0.02.

perfusate VLDL triglyceride from the homologous rats, the most noticeable feature was the significantly higher content of oleate in the triglyceride secreted into the perfusate.

Similar differences in the fatty acid composition of serum triglycerides were observed in palmitate and linoleate (Table 4). The oleate content of serum triglyceride was also increased in the obese animals. The comparison of serum triglyceride and homologous perfusate VLDL triglyceride indicated approximately half as much oleate and twice as much linoleate in the serum triglyceride.

To investigate the relative susceptibility of the lean and obese rat liver perfusate VLDL to lipolysis, we utilized a highly purified human milk LPL. By adjusting both hepatic VLDL preparations to an initial triglyceride concentration of 0.3 mg/ml, we initiated the lipolysis by using an identical quantity of LPL. Both of the perfusate VLDL preparations appeared to have sufficient amounts of cofactor (apoC-II), because the addition of serum to the reaction medium did not enhance the rate of lipolysis. During lipolysis, the TG-50/TG-54 (see Materials and Methods) was found to maintain a constant ratio, which suggests no preferential accumulation of a particular triglyceride molecular species during lipolysis. The kinetic pattern of triglyceride disappearance is shown in **Fig. 4.** During the initial 50% of lipolysis, the rate of triglyceride degradation can be described as following pseudo-first order kinetics. The rate constant k_1 was determined for obese rat hepatic VLDL as 0.073 min^{-1} ± 0.010 (SD) and a value of 0.060 min^{-1} ± 0.010 (SD) for lean rat hepatic VLDL was obtained. Thus, the relative rates were 1.22:1.00 for the lipolysis of obese versus lean rat liver perfusate VLDL by an identical amount of LPL. There was no statistically significant difference in these rates. Therefore, although there were some specific differences in fatty acid composition, there was no retardation of lipolysis of the triglyceride in VLDL particles secreted by the obese Zucker rat liver.

DISCUSSION

Previous studies by Schonfeld and Pfleger (6) and from this laboratory (7) have demonstrated hypersecretion of triglyceride-rich lipoproteins by livers of obese Zucker rats. In the present study, we have examined the possibility that impaired removal of these plasma lipoproteins from the circulation may contribute to the marked hypertriglyceridemia in these animals. LPL, a major determinant in the removal process (8–11), in the tissues of lean and obese Zucker rats was examined. Because functional LPL resides on the luminal surface of the capillary endothelium, we assayed heparin-released LPL activity rather than the activities in tissue homogenates.

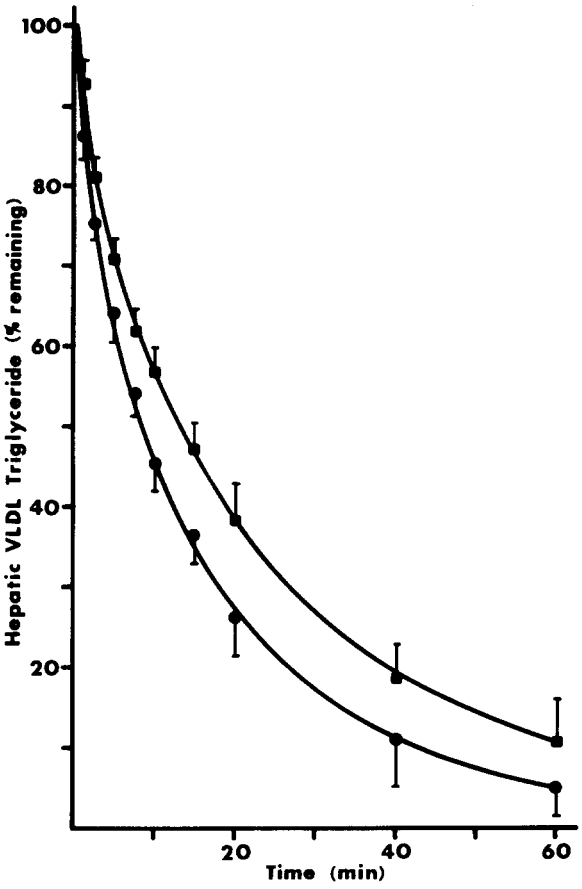

Fig. 4. Kinetics of the degradation of hepatic VLDL-triglyceride, isolated from the liver perfusates from lean (■) and obese (●) Zucker rats, catalyzed by human milk LPL. The initial triglyceride concentration was adjusted to 0.3 mg/ml.

We found no significant difference in the heparin-released LPL activities of adipose tissue, skeletal muscle, and heart (expressed per gram of tissue) from the lean versus obese Zucker rats. In this assay, lipase activity could only be detected in the presence of the cofactor, which was supplied by serum. This suggests that, during the release of LPL by heparin treatment, tissue cellular integrity was maintained to account for the lack of intracellular lipase released during our experimental manipulations. Previous study by Gruen, Hietanen, and Greenwood (12), using the postmitochondrial supernatant fraction of adipose tissue and with enzyme activity expressed as per gram weight of tissue, indicated that LPL is greatest in the young rats and there is no significant difference between obese and lean Zucker rats. The study by Hartman (13), using an enzyme preparation from the supernatant fraction of tissue homogenates, indicated that LPL activity from adipose tissue, adipocytes, heart, and skeletal muscle at 9–11 and 26–30 weeks of age, also showed no difference between lean and obese Zucker rats. Despite the difference in the procedures of enzyme preparation, there is a general agreement that the total LPL activities (per unit weight of tissue) in lean and obese rats are similar.

In this study, we observed no difference between lean and obese Zucker rats in the LPL kinetic behavior from the heparin extract of adipose tissue and heart (Fig. 3). Similar to our finding, crude homogenates of lean and obese Zucker rat adipose tissue exhibited the same K_m (12). We observed that the kinetic properties of heart LPL from both lean and obese Zucker rats were not in conformity with Michaelis-Menten kinetics (Fig. 3). Therefore, a K_m value could not be assigned for these LPL activities. However, based on their similar sigmoidal response to substrate concentration, we conclude that heart LPL from lean and obese animals is similar if not identical. The previous report (23) of a low K_m value for rat heart LPL (0.07 mM) and a high K_m value (0.7 mM) for rat adipose tissue was derived from an indirect measurement using tissue perfusion procedures. The studies of Ben-Zeev, Schwalb, and Schotz (24) using Sprague-Dawley rats suggest the presence of two types of LPL in heart tissue. The LPL in the heparin perfusate and in the residue were partially purified by heparin-Sepharose affinity and found to have a significant difference in apparent K_m values for triglyceride. The reason for the abnormal kinetic behavior of LPL observed in the present study in the heparin extract of rat heart is unknown. Several possibilities have been considered at present: *1*) heterogeneity of LPL in the heparin extract of heart tissue; *2*) a modulator factor in the heparin extract; *3*) the sigmoidal response may be due to an intrinsic property of LPL. Determination of the actual molecular basis for this behavior will await future study of the purified enzyme. Regardless of the actual explanation, the fact that LPL from adipose tissue and heart under identical assay conditions exhibited different kinetic properties clearly supports the suggestion that LPL from different tissues may have different properties (10). This has important implications in the regulation of the plasma triglyceride level and deserves a further detailed comparative study.

Similar to heparin-releasable tissue LPL, there was also no significant difference in postheparin plasma LPL activities of lean and obese Zucker rats (Tables 1 and 2). Thus, the heparin-releasable tissue LPL and postheparin plasma LPL activities correlated. However, for the assessment of removal of triglyceride from the plasma, the measurement of plasma postheparin LPL might be preferred since this activity represents the total heparin-accessible tissue LPL released into the blood compartment, rather than the activity derived from a single specific tissue. In contrast to the finding of similar activity of LPL in postheparin plasma of lean and obese rats, we observed a significantly higher activity of H-TGL in obese rats. It is apparent that the higher H-TGL could not alleviate the hypertriglyceridemia in the obese rats, possibly be-

cause the in vivo accumulated triglyceride-rich lipoproteins were poor substrates for H-TGL. The mechanism of higher H-TGL in obese rats is not clear. One possible mechanism is that its activity was induced in liver (25) by the hyperinsulinemia in obese Zucker rats (12, 26). Another possible mechanism is that its activity was induced in the liver in response to hypertriglyceridemia. We are currently investigating these and other possible mechanisms.

From the measurement of LPL activity and examination of kinetic properties of LPL, we have concluded that the level and functional properties of LPL were not responsible for the hypertriglyceridemia in obese Zucker rats. However, because the rate of lipolysis is not dependent on the enzyme alone, we also examined the possible contribution of retarded lipolysis of the substrate due to an abnormality in the triglyceride-rich lipoproteins secreted by the liver. These studies demonstrated differences in fatty acid composition of triglyceride in VLDL secreted by livers from lean and obese animals. There was a higher percent content of palmitate and lower content of linoleate in secreted VLDL from the obese rat liver. This difference probably reflects enhanced hepatic synthesis of palmitate by the liver of the obese genotype (27, 28). The fatty acid composition of perfusate triglyceride reflected that of liver triglyceride, except for oleate which was enriched in the hepatic VLDL. This is the result of the use of oleate substrate in the liver perfusion and the preferential secretion of the free fatty acid substrate in newly synthesized VLDL particles (7). Accordingly, the percentages of oleate in the triglyceride secreted by lean and by obese rat livers were similar (Table 4). Despite the differences in fatty acid composition of VLDL, the VLDL from the lean and obese rats exhibited similar rates of lipolysis. The VLDL from both lean and obese rat liver perfusates contained sufficient amounts of functionally active cofactor apolipoprotein C-II, based on the finding that the addition of serum to the lipolysis mixture did not enhance the rate of lypolysis. The red blood cells in the perfusion medium were washed three times and were devoid of apolipoprotein C-II activity. The kinetics of VLDL-triglyceride degradation were found to be in conformity with pseudo-first order kinetics when the lipolysis is less than 50%. This is probably due to the lack of product inhibition in this period. From this study, we have concluded that there is no abnormality in the structure of the VLDL particles secreted by the obese rat liver, at least in terms of its lipolytic degradation by LPL.

Previous studies have clearly demonstrated the hypersecretion of triglyceride-rich lipoproteins by the obese Zucker rat liver (6, 7). In the present study, we have provided evidence for a normally functional lipolytic system in the obese rat. Therefore, we have concluded that the hypertriglyceridemia of the obese Zucker rat is primarily due to the hepatic hypersecretion of triglyceride-rich lipoproteins which exceeds the capacity of the lipolytic system in the initial step of the removal process. Redgrave (5) observed that partially degraded chylomicron particles accumulate in the plasma of these animals. The prolonged half-life of chylomicron triglyceride concurrent with increased plasma triglyceride turnover (5) is entirely consistent with near saturation of LPL-catalyzed triglyceride removal. The increased food intake of the obese Zucker rat may accordingly exacerbate the hyperlipemic condition.

The excellent technical assistance of Helen B. Bass and Deborah Downs is gratefully acknowledged. This work was supported by Grants HL 23181 and HD 14104 from the United States Public Health Service.

Manuscript received 22 August 1983.

REFERENCES

1. Wang, C-S., and J. A. Ontko. 1983. Lipolysis of very low density lipoproteins from lean and obese Zucker rat liver perfusates. *Federation Proc.* **42:** 2017 (Abstract).
2. Zucker, T. F., and L. M. Zucker. 1962. Hereditary obesity in the rat associated with high serum fat and cholesterol. *Proc. Soc. Exp. Biol. Med.* **110:** 165–171.
3. Barry, W. S., and G. A. Bray. 1969. Plasma triglycerides in genetically obese rats. *Metabolism.* **18:** 833–839.
4. Schonfeld, G., C. Felski, and M. A. Howald. 1974. Characterization of the plasma lipoproteins of the genetically obese hyperlipoproteinemic Zucker fatty rat. *J. Lipid Res.* **15:** 457–464.
5. Redgrave, T. G. 1977. Catabolism of chylomicron triacylglycerol and cholesteryl ester in genetically obese rats. *J. Lipid Res.* **18:** 604–612.
6. Schonfeld, G., and B. Pfleger. 1971. Overproduction of very low-density lipoproteins by livers of genetically obese rats. *Am. J. Physiol.* **220:** 1178–1181.
7. Fukuda, N., M. J. Azain, and J. A. Ontko. 1982. Altered hepatic metabolism of free fatty acids underlying hypersecretion of very low density lipoproteins in the genetically obese Zucker rat. *J. Biol. Chem.* **257:** 14066–14072.
8. Robinson, D. S. 1970. The function of the plasma triglycerides in fatty acid transport. *In* Comprehensive Biochemistry. M. Florkin and E. H. Stotz, editors. Elsevier, Amsterdam. 51–116.
9. Kompiang, I. P., A. Bensadoun, and M-W. W. Yang. 1976. Effect of antilipoprotein lipase serum on plasma triglyceride removal. *J. Lipid Res.* **17:** 498–505.
10. Fielding, C. J., and R. J. Havel. 1977. Lipoprotein lipase. *Arch. Pathol. Lab. Med.* **101:** 225–229.
11. Nilsson-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49:** 667–693.
12. Gruen, R., E. Hietanen, and M. R. C. Greenwood. 1978. Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa). *Metabolism.* **27:** 1955–1966.
13. Hartman, A. D. 1981. Lipoprotein lipase activities in adipose

tissues and muscle in the obese Zucker rat. *Am. J. Physiol.* **241:** E108–E115.
14. Schotz, M. C., A. S. Garfinkel, R. J. Huebotter, and J. E. Stewart. 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.* **11:** 68–69.
15. Wang, C-S., D. Weiser, P. Alaupovic, and W. J. McConathy. 1982. Studies on the degradation of human very low density lipoproteins by human milk lipoprotein lipase. *Arch. Biochem. Biophys.* **214:** 26–34.
16. Kuusi, T., C. Ehnholm and E. A. Nikkilä. 1980. Immunochemical assay of rat postheparin plasma triacylglycerol lipases. *Atherosclerosis.* **35:** 363–374.
17. Wang, C-S., H. B. Bass, D. Downs and K. Witmer. 1981. Modified heparin-Sepharose procedure for determination of plasma lipolytic activities of normolipidemic and hyperlipidemic subjects after injection of heparin. *Clin. Chem.* **27:** 663–668.
18. Wang, C-S., and R. L. Smith. 1975. Lowry determination of protein in the presence of Triton X-100. *Anal. Biochem.* **63:** 414–417.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
20. Carey, C., C-S. Wang, and P. Alaupovic. 1975. A critical evaluation of the proposal that serum apolipoproteins are the major constituents of the human erythrocyte membrane. *Biochim. Biophys. Acta.* **401:** 6–14.
21. Wang, C-S. 1981. Human milk bile salt-activated lipase. Further characterization and kinetic studies. *J. Biol. Chem.* **256:** 10198–10202.
22. Wang, C-S., A. Kuksis, and F. Manganaro. 1982. Studies on the substrate specificity of purified human milk lipoprotein lipase. *Lipids.* **17:** 278–284.
23. Fielding, C. J. 1976. Lipoprotein lipase: evidence for high- and low-affinity enzyme sites. *Biochemistry.* **15:** 879–884.
24. Ben-Zeev, O., H. Schwalb, and M. C. Schotz. 1981. Heparin-releasable and non-releasable lipoprotein lipase in the perfused rat heart. *J. Biol. Chem.* **256:** 10550–10554.
25. Nakai, T., S. Yamada, T. Tamai, T. Kobyashi, T. Hayashi and R. Takeda. 1979. The effects of streptozotocin diabetes on hepatic triglyceride lipase activity in rat. *Metabolism.* **28:** 30–40.
26. Zucker, L. M., and H. N. Antoniades. 1972. Insulin and obesity in the Zucker genetically obese rat "fatty". *Endocrinology.* **90:** 1320–1330.
27. Martin, R. J. 1974. In vivo lipogenesis and enzyme levels in adipose and liver tissues from pair-fed genetically obese and lean rats. *Life Sci.* **14:** 1447–1453.
28. Sullivan, A. C., J. Triscari, and H. E. Spiegel. 1977. Metabolic regulation as a control for lipid disorders. *Am. J. Clin. Nutr.* **30:** 777–784.