

Very low density lipoproteins and lipoprotein lipase in serum of rats deficient in essential fatty acids

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Abstract Rats fed a diet deficient in essential fatty acids have a low level of serum very low density lipoproteins (VLDL). It was found that after intraperitoneal injection of heparin, deficient rats had a higher level of lipoprotein lipase activity in their plasma than did normal rats. VLDL isolated from serum of normal and deficient rats were compared as substrates for postheparin lipase of rat plasma. There was no significant difference in V_{max} between the two preparations of lipoproteins, but the apparent K_m for lipoproteins from deficient animals was significantly less than that for normal animals. These observations suggest that the low concentration of VLDL in deficient rats may be explained (a) by an increased activity of lipoprotein lipase in the tissues of these animals and (b) by the VLDL of deficient rats being more rapidly hydrolyzed at low concentrations by lipoprotein lipase than VLDL from normal rats.

Supplementary key words fatty acid composition · phospholipids · triglycerides · cholesteryl esters · heparin · enzyme kinetics

THERE IS a well established dietary requirement for essential fatty acids (EFA), and among the many signs of EFA deficiency are alterations in the fatty acid composition of tissue lipids, development of a fatty liver, and changes in the levels of serum lipoproteins (1–4). Sinclair and Collins (4) have shown that rats deficient in EFA have a higher than normal content of triglyceride in their livers, but a lower than normal level of blood

lipids. This reduction in the level of plasma lipids is most marked in the VLDL fraction. However, an inhibition of lipid secretion from liver to plasma is not a cause of these events, because the rate of this secretion in deficient rats is slightly higher than in normal animals (4). It thus appears that although the flux of triglyceride from liver to plasma is greater in deficient animals, the steady state concentration of triglyceride in the plasma is lower than normal. There must be a more rapid removal of triglyceride from plasma of deficient rats.

A major pathway in the removal of triglyceride from plasma is by the action of lipoprotein lipase in the capillaries of extrahepatic tissue to produce free fatty acids, which are then absorbed into the tissue (5). It was decided, therefore, to investigate the level of lipoprotein lipase in deficient rats and to compare the effects of this enzyme on VLDL from normal and deficient animals.

MATERIALS AND METHODS

Animals

The rats used in these experiments were males of an inbred strain of Sprague-Dawley rats obtained from Holtzman's Rat Farm, Madison, Wis. After weaning at between 19 and 24 days of age, they were fed either a stock diet of dog cubes (6) or a synthetic fat-free diet supplemented with 5% hydrogenated beef fat (4). After 14 wk the animals on the synthetic diet showed all the symptoms of a deficiency of EFA (4, 7). These latter rats will be referred to in this paper as deficient rats and those on the stock diet as normal rats. The rats had continuous access to food and water and they were not fasted prior to being killed.

Abbreviations: VLDL, very low density lipoproteins; PL, phospholipids; TG, triglycerides; CE, cholesteryl esters; EFA, essential fatty acids; FFA, free fatty acids.

The choline content of the synthetic diet is 0.0375% plus the metabolic equivalent of choline in the 25% of casein in the diet. It has been suggested (8) that this level is suboptimal and that it could be responsible for the accumulation of fat in the livers of our deficient rats. However, supplementing the diet with extra choline to raise the level to 0.1%, as recommended by Holman (2), did not prevent the appearance of a low level of serum lipids accompanied by an elevated level of liver fat,¹ although the amount of liver fat in rats on this diet was not as great as previously reported (4).

Preparation of postheparin plasma

Heparin was administered intraperitoneally rather than intravenously because it was difficult to inject an accurate dose to deficient rats due to the scaliness of their tails. Rats were anesthetized with diethyl ether, injected with heparin, and then allowed to recover. After the appropriate time, usually 60 min, the rats were again anesthetized then decapitated, and the blood was collected in graduated centrifuge tubes containing 0.5 ml of 0.1 M sodium citrate. Additional citrate solution was added immediately so that the final concentration in the blood was 0.01 M. The tubes were chilled in ice for a few minutes before centrifuging at 600 g for 15 min at 5°C. The resulting plasma was kept at 0°C for immediate assay, or it was frozen and stored at -20°C. It was found that lipase activity was unaffected by freezing and storage for several days; therefore, all lipase measurements were routinely made on plasma that had been frozen. The lipase activities reported in this paper refer to the rates of lipolysis per milliliter of citrated plasma.

Preparation of VLDL

Rats were anesthetized with diethyl ether and killed by decapitation; the blood was collected, pooled, and allowed to clot. After centrifuging, the serum was collected and 5% EDTA (pH 7.0) was added so that the final concentration in the serum was 0.05%. Chylomicrons were removed by centrifuging at 9500 g for 30 min in a Spinco model L preparative ultracentrifuge. VLDL ($d < 1.006$) were then separated as described by Havel, Eder, and Bragdon (9). The VLDL were washed once with 0.15 M NaCl containing 0.05% EDTA and stored at 5°C. VLDL were used in lipase experiments within 24 hr of their preparation. Samples of VLDL for chemical analysis were frozen and stored at -20°C until analyzed.

Chemical analyses

Lipid phosphorus of VLDL was determined after extracting samples with chloroform-methanol 2:1 (v/v)

¹ Collins, F. D., and S. E. Rands. Unpublished results.

by the method of Folch, Lees, and Sloane Stanley (10), as modified by Fleischer, Klouwen, and Brierley (11), and estimating phosphorus in the washed extract (12). The molecular weight of phospholipid was taken as 800. Total cholesterol was measured by the method of Searcy and Bergquist (13). Free cholesterol was calculated from this figure by subtracting the cholesterol equivalent of cholesteryl esters, determined by gas-liquid chromatography as described below.

Total lipids were extracted from freeze-dried samples of VLDL and separated by thin-layer chromatography, as described by Sinclair and Collins (4). The triglyceride, cholesteryl ester, and phospholipid spots were scraped from the plates quantitatively, and their fatty acids were analyzed by gas-liquid chromatography of the methyl esters, using a known amount of methyl arachidate as an internal standard (14). The ratios of triglyceride and of cholesteryl ester to phospholipid were calculated and hence the concentration of triglyceride and cholesteryl esters in the VLDL. Protein was measured by a slight modification of the method of Lowry et al. (15). Turbidity caused by high concentrations of lipids was removed by extraction with diethyl ether at room temperature after the color had fully developed (16).

The level of heparin in blood was measured as described by Jacques and Bell (17) by titrating with standardized protamine sulfate solution (Boots Pure Drug Co., Nottingham, England) until a minimum clotting time was obtained.

Chemicals

Bovine serum albumin (Cohn fraction V) was obtained from the Commonwealth Serum Laboratories, Parkville, Victoria. Intralipid, a commercial emulsion of soybean triglycerides, was manufactured by Vitrum AB, Stockholm, Sweden. Heparin (Drug Houses of Australia Pty. Ltd.) was diluted with 0.15 M NaCl before injection. Doses are expressed in international units/kg body weight. Other chemicals were of analytical reagent grade and all solvents were redistilled before use.

Measurement of lipoprotein lipase activity

Lipase activity expressed as μ moles of fatty acids released per min per ml of citrated plasma was measured in a pH stat (Radiometer, Copenhagen) at pH 8.0 and 37°C by titrating the acid released after the addition of substrate (VLDL or Intralipid) to an incubation mixture containing postheparin plasma. The incubation mixture consisted of 1.0 ml of 20% bovine serum albumin, pH 8.0, 1.0 ml of postheparin plasma, substrate, and 0.15 M sodium chloride to a final volume of 5 ml. The titration was carried out under an atmosphere of nitrogen, using 0.02 M or 0.04 M sodium hydroxide. Acid production commenced within half a minute of

adding substrate and there was no production of acid before adding substrate. Blank rates of acid production, measured by replacing plasma with 0.15 M sodium chloride, were less than 5% of experimental rates and were subtracted from the observed rates. Consistent rates of acid production were not established until about 1 or 2 min after the reaction was initiated. Hence, the initial rate of reaction was measured 2.5 min after the start of lipolysis. After several minutes the rate of acid production became progressively slower in some experiments. This was more marked with VLDL as substrate than with Intralipid and was probably due to depletion of substrate.

To check that the proton release measured by the pH stat was due to the production of FFA from lipolysis, the amount of fatty acids calculated from titration was compared with the amount of FFA extracted from a duplicate incubation by the procedure of Dole (18) and assayed as described by Novák (19). Over the first 4 min of incubation there was reasonable agreement between the two methods (Fig. 1).

Benzonana and Desnuelle (20) have investigated the use of a pH stat to measure lipolytic activity of pancreatic lipase. They found that under some conditions corrections needed to be made for the incomplete ionization of the FFA formed due to their occurrence as micelles. However, this correction affects the value of absolute rates but not the relative value of rates of lipolysis measured under identical conditions. Moreover, agreement between rates determined by pH stat

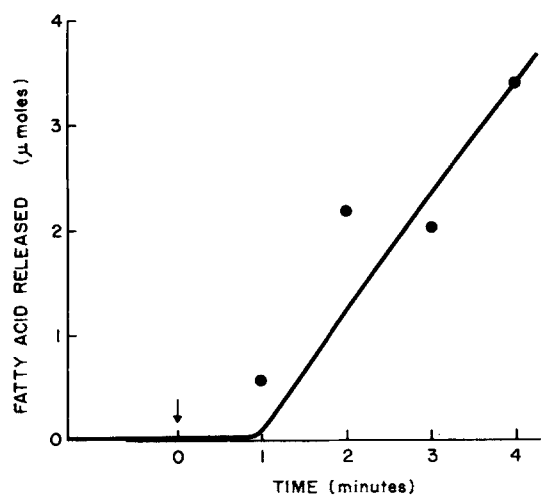


FIG. 1. Comparison of production of FFA during lipolysis as measured by pH stat and by extraction. 1.0 ml of postheparin plasma from a normal rat was incubated at 37°C, pH 8.0, with a mixture of 1.0 ml of 20% albumin and 2.4 ml of 0.15 M sodium chloride. At the time shown by the arrow, 0.6 ml of Intralipid (200 mg of TG/ml) was added. The solid line shows the amounts of FFA released as measured by the pH stat. The solid circles show the amounts of FFA released as measured after extraction of a duplicate incubation mixture.

and those measured by titration (Fig. 1) shows that under our conditions this correction would be small. Our system, unlike that of Benzonana and Desnuelle (20), contained albumin as an acceptor for the FFA, thus preventing the formation of micelles.

RESULTS

Composition of VLDL

The concentration of VLDL in the serum of deficient rats was less than half of that in normal rats (Table 1). The VLDL from both groups of rats contained the same proportions of triglycerides and of free cholesterol plus cholesteryl esters. The VLDL from deficient animals had a slightly higher ratio of cholesteryl esters to free cholesterol. The proportion of protein plus phospholipid was also the same for VLDL from both groups of animals, but the deficient rats had more protein and correspondingly less phospholipid than normal.

Fatty acid analysis of the lipids (Table 2) shows the usual marked difference that is found between the fatty acids of normal animals and those deficient in essential fatty acids. The linoleic and arachidonic acids in the VLDL of normal animals were replaced in the deficient animals by palmitoleic, oleic, and 5,8,11-eicosatrienoic acids. This change occurred equally in all three lipid fractions. The proportions of the saturated fatty acids were not altered significantly.

Lipoprotein lipase activities in postheparin plasma of normal and deficient rats

Using Intralipid as a substrate, no lipoprotein lipase activity could be detected in the plasma of either normal or deficient rats that had not been injected with heparin. After injection with heparin, lipase activity could be measured in the plasma and the level of activity was constant between 40 and 100 min after injection (Table 3). In subsequent experiments, animals were killed 60 min after injection with heparin.

TABLE 1. Composition of VLDL in the serum of normal and deficient rats

	Normal	Deficient
	<i>mg/100 ml of serum</i>	
Protein	10.1 (9.5)	5.7 (11.5)
PL	14.5 (13.6)	5.6 (11.3)
TG	72.8 (68.1)	33.8 (68.5)
CE	5.3 (4.9)	2.8 (5.6)
Cholesterol	4.1 (3.9)	1.5 (3.1)
Total	106.8 (100)	49.4 (100)

VLDL were prepared from the pooled serum of 28 normal rats and 32 deficient rats. The figures in parentheses show percentage composition.

TABLE 2. Fatty acid composition of the lipids in VLDL of normal and deficient rats

Fatty Acid	Normal			Deficient		
	PL	TG	CE	PL	TG	CE
	% of total fatty acids					
14:0 ^a	0.3	2.1	0.5	0.3	1.6	1.1
16:0	17.2	21.5	12.9	17.8	26.3	14.8
16:1	0.7	3.4	4.6	2.2	11.9	15.9
18:0	23.8	6.0	10.0	25.0	5.3	7.0
18:1	11.9	37.9	28.7	25.3	50.2	38.5
18:2	22.8	19.6	16.7	1.3	0.8	2.5
18:3	0.4	1.3	0.7	0.6	0.9	0.7
20:3(n - 9)	0.3	0.3	0.7	16.8	0.8	9.5
20:3(n - 6)	1.5	0.2	<0.1	1.8	<0.1	<0.1
20:4	12.0	1.7	19.5	2.3	<0.1	3.7
22:3	1.8	<0.1	<0.1	1.4	<0.1	<0.1
22:4(n - 6)	2.0	0.2	<0.1	1.6	<0.1	<0.1
22:6	2.2	<0.1	<0.1	0.8	<0.1	<0.1

Fatty acids occurring with an abundance of less than 1% have not been included in the table.

^a Number of carbon atoms: number of double bonds.

TABLE 3. Plasma lipoprotein lipase activity after intraperitoneal injection of heparin

Time after Injection	Lipase Activity
min	$\mu\text{moles FFA}/\text{min}/\text{ml}$
20	0.69
40	1.07
60	1.03
80	1.22
100	1.14

Five normal rats were injected intraperitoneally with 3500 units/kg body wt of heparin at different times before they were killed. Lipase activity in the plasma was measured at 37°C and pH 8.0 using Intralipid (24 mg of TG/ml) as substrate.

When normal and deficient rats were treated with increasing intraperitoneal doses of heparin, it was found that the level of lipase activity in their plasma increased with increasing heparin dose until a plateau of maximum response was reached (Fig. 2). This occurred at a heparin dose of approximately 2500 units/kg in normal animals and 2000 units/kg in deficient animals. Larger doses of heparin did not cause any further increase in lipase activity. The maximum lipase activity released by heparin into the plasma of deficient rats was approximately twice that of normal rats.

This difference in lipase activity between normal and deficient rats was not due to heparin being more readily absorbed from the intraperitoneal cavity in deficient rats. Measurement of levels of heparin in blood after intraperitoneal injection showed that the concentration in both normal and deficient rats was similar for equivalent doses of heparin (Table 4). Moreover, even though increasing the heparin dose from 3000 to 4000 units/kg increased the level of heparin in the blood (Table 4), it did not produce any increase in lipase

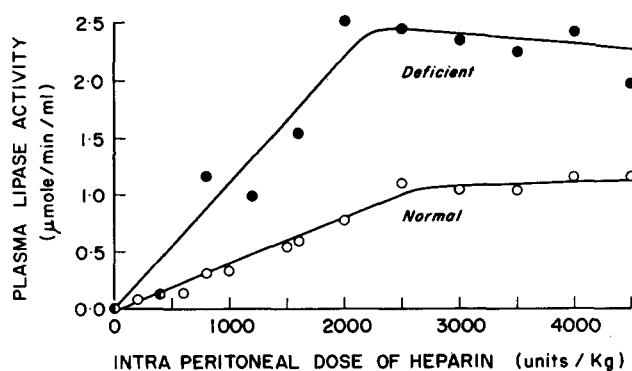


FIG. 2. Plasma lipoprotein lipase activity after increasing intraperitoneal doses of heparin. Normal and deficient rats were injected with heparin 60 min before they were killed. Lipase activity was measured at 37°C and pH 8.0, using Intralipid (24 mg of TG/ml) as substrate. Each point represents the result obtained with one animal.

activity (Fig. 2). Thus, with high doses of heparin, plasma lipase activity is at a maximum which is independent of the level of heparin in the blood.

Comparison of VLDL from normal and deficient rats as substrate for lipoprotein lipase

A comparison was made of the effectiveness of VLDL from normal and deficient rats as substrates for the enzyme. With both types of VLDL, lipase activity was completely inhibited in the presence of 1 M sodium chloride. The response of lipase activity with increasing concentrations of substrates appeared to obey Michaelis-Menten kinetics (Fig. 3). It can be seen that while the maximum rate of lipolysis (V_{max}) for each substrate was similar, they differed in the value of their apparent Michaelis constants (K_m). Values of K_m and V_{max} were calculated by using a computer program (21) to fit the experimental data for rate of lipolysis (v) and concentration of substrate (c) to the Michaelis-Menten equation: $v = (V_{max}c)/(K_m + c)$. Values obtained for normal and deficient VLDL and also for Intralipid are shown in Table 5. There was no significant difference between the values for V_{max} . However, K_m for VLDL from deficient rats was significantly less than that from

TABLE 4. Level of heparin in blood 60 min after intraperitoneal injection

Heparin Injected	Heparin in Blood	
	Normal	Deficient
units/kg	units/ml	
3000	4.3	4.3
3500	4.3	5.1
4000	5.4	6.3

Three normal and three deficient rats were injected with heparin 60 min before they were killed.

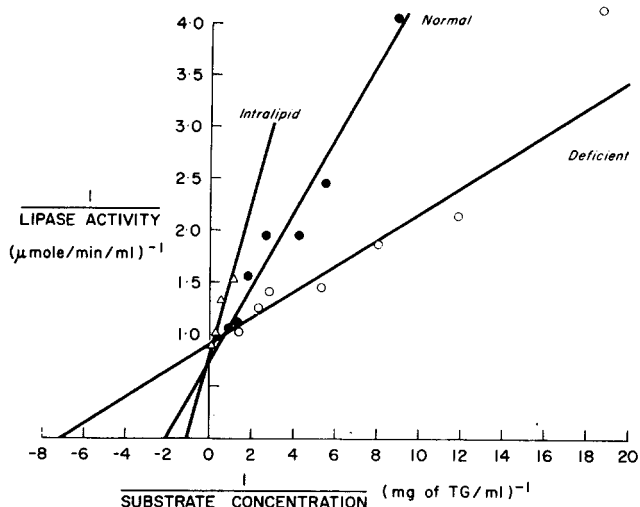


FIG. 3. Lineweaver-Burk plot of plasma lipase activity with increasing concentration of substrate. Aliquots of pooled postheparin plasma from normal rats were used to assay lipase activity at 37°C, pH 8.0. The substrates used were Intralipid and VLDL from normal and deficient rats. The same pooled sample of plasma was used with all substrates.

normal rats. K_m for Intralipid was greater than that of either of the VLDL.

Comparison of lipases from normal and deficient rats

As a check to ensure that the lipase in the plasma of deficient rats had the same kinetic characteristics as that in the normal rats, a comparison of the two enzymes was made using various concentrations of Intralipid as a common substrate. The experimental data was fitted to the Michaelis-Menten equation and V_{max} and K_m calculated as above. The results (Table 6) show that, as expected, V_{max} for plasma from deficient rats was significantly greater than that for normal animals. However, the values of K_m for the lipase from normal and deficient rats did not differ significantly from each other. These results indicate that the lipase activity of postheparin plasma from deficient rats differs from that in normal rats only in as much as its concentration is higher.

TABLE 5. Comparison of serum VLDL from normal and deficient rats as substrates for plasma lipoprotein lipase

Substrate	V_{max}	K_m
	$\mu\text{moles FFA}/\text{min}/\text{ml}$	$\text{mg of TG}/\text{ml}$
Normal VLDL	1.34 (1.14–1.54)	0.48 (0.29–0.80)
Deficient VLDL	1.11 (0.97–1.25)	0.14 (0.07–0.25)
Intralipid	1.25	0.94

These values are calculated from the data shown in Fig. 3. The figures in parentheses are confidence limits at the 5% level for V_{max} and at the 1% level for K_m (21).

TABLE 6. Comparison of lipoprotein lipase from plasma of normal and deficient rats using Intralipid as substrate

Lipase	V_{max}	K_m
	$\mu\text{moles FFA}/\text{min}/\text{ml}$	$\text{mg TG}/\text{ml}$
Normal	0.93 (0.87–1.00)	0.76 (0.57–1.00)
Deficient	1.61 (1.42–1.79)	0.99 (0.64–1.53)

Aliquots of pooled postheparin plasma from normal rats and from deficient rats were used to assay lipase activity with 10 different concentrations of Intralipid at 37°C, pH 8.0. The experimental data obtained were fitted to the Michaelis-Menten equation using a computer program (21) to give values for K_m and V_{max} . The figures in parentheses are confidence limits at the 5% level.

DISCUSSION

It has been observed previously in this laboratory that rats fed a synthetic control diet have a higher level of serum triglycerides than do rats fed dog cubes (22). Hence it is not surprising that the level of VLDL in the serum of normal rats in these experiments is lower than some of those reported by Sinclair and Collins (4) for rats fed a control diet. But the level of VLDL reported here is considerably higher than that observed by Lombardi and Ugazio (23), who used younger animals that had previously been starved for 18 hr. This period of starvation, which greatly reduces the concentration of serum triglycerides in rats (4), probably explains the low values they obtained. The composition of the VLDL is similar to the ranges of compositions which have been reported recently for normal rats (24–26). Differences among the analyses from various laboratories are probably due to differences in diet and in the severity of starvation before killing.

The deficient rats had a much lower level of VLDL in their serum, confirming previous observations (4). The proportion of triglyceride was the same in VLDL from both normal and deficient animals, but as observed previously (4), in VLDL from deficient rats the ratio of phospholipid to triglyceride was lower and that of protein to triglyceride higher than normal. In addition, there was in the deficient animal an increase in the proportion of esterified cholesterol. However, in VLDL from both groups of animals the ratios of nonpolar components (triglyceride, cholesterol, and cholesteryl esters) to polar components (phospholipid and protein) were the same. It appears that the ratio of hydrophobic core to hydrophilic surface material is the same in VLDL from both normal and deficient rats, but the deficient animals replace some phospholipid by protein and esterify more of the cholesterol than normal animals.

The discovery that rats deficient in EFA have a lowered level of serum lipids coupled with an increased amount of fat in their livers (1–4) suggests an impairment in the secretion of fat from liver to serum. Such an

impairment in perfused livers of deficient rats has been reported by Fukazawa, Privett, and Takahashi (8). However, in our laboratory, *in vivo* experiments using Triton to block the removal of triglycerides from serum show that the rate of secretion of serum triglycerides by liver in deficient rats is not inhibited compared with normal controls (4); in fact, these experiments suggest there may be a moderate increase in the deficient animals. The value obtained in these Triton experiments of 0.33 mg/min/100 g body weight for the rate of secretion of triglyceride from liver of normal rats is in good agreement with the value of 0.26 mg/min/100 g body weight calculated by Baker and Schotz (27) from the rate of change of radioactivity of lipids following a single intravenous injection of [¹⁴C]palmitate. Calculation from the data of Fukazawa et al. (8) gives a value for the rate of triglyceride secretion from the perfused livers of these control rats of only 0.04 mg/min/100 g body weight. This low value may be the result of a limiting amount of FFA in the perfusion medium, but it also suggests that in the perfused livers the secretory mechanism was damaged. Moreover, during perfusion, livers from control animals accumulated a large amount of triglyceride; about 75% of the mass of FFA taken up by the livers was retained as liver triglyceride and phospholipid. This is in marked contrast to the situation *in vivo*, where the lipid content of the liver is maintained at an equilibrium level in fed animals, and indicates an impairment of secretion from perfused livers. The sensitivity of enzyme systems in deficient rats to damage during isolation is well known (2, 7, 28, 29). Hence it is not surprising that Fukazawa et al. (8) found that in the perfused livers of deficient rats the rate of triglyceride secretion was even more impaired than it was in livers of normal rats. However, the results of these perfusion experiments cannot be taken to show that there is an actual impairment of secretion *in vivo* in the livers of deficient animals. Thus, the problem remains to explain the low level of serum lipids in deficient rats when Triton experiments indicate that the output of triglycerides from livers of these rats into their serum is at least as great as in normal animals (4).

The value we obtained for lipoprotein lipase activity in the postheparin plasma of normal rats is higher than that reported by Salaman and Robinson (30), but this is probably due to the higher doses of heparin and the different substrate and assay conditions used in our experiments.

The postheparin lipase activity in deficient rats is double that of normal animals, and this could be caused by either a lowered rate of lipase removal or an increase in lipase, released by heparin, in the tissues. There have been reports that rats with livers damaged by partial hepatectomy or by carbon tetrachloride have a higher postheparin lipase activity (31) due to the loss of the

ability of the liver to remove lipase activity from the blood (5, 32). It is conceivable that the fatty livers of deficient rats (4) may be sufficiently damaged to impair their ability to remove lipase. However, this removal of lipase activity by liver is not observed in normal rats after high doses of heparin, when lipase activity is at a peak (32, 33). It can only be detected when lipase activity in the plasma is low. Our rats were treated with doses of heparin that induced maximum lipase activity which was measured at the optimum time after injection. Under these conditions the normal liver would not have the capacity to lower significantly the observed level of plasma lipase activity. Moreover, the livers of deficient rats are not drastically damaged as are those in animals poisoned with carbon tetrachloride. In these poisoned livers, damage is such that lipoprotein secretion is impaired, but no impairment of lipoprotein secretion is found in the livers of deficient rats (4).

There is much evidence that tissue lipoprotein lipase is responsible for removal of lipids from blood (5, 34) and that the level of tissue lipase activity can be correlated with plasma triglyceride levels (35, 36). The level of lipase in postheparin plasma has also been correlated with the level of lipase in the tissues (30). Moreover, Borensztajn and Robinson (37) concluded "that correlations between triglyceride fatty acid utilization and clearing factor lipase activity in the heart should be sought only with that portion of the total enzyme activity which is released from the intact organ by heparin." Hence, the lipase activity measured after the administration of heparin will represent the total quantity of enzyme available. Since we used larger doses of heparin and obtained a maximum release of lipase activity into the plasma of both normal and deficient rats, our results strongly suggest that there is more lipoprotein lipase available in the tissues of deficient rats than in normal animals. This could be because deficient rats have more enzyme present or that it is more readily released by heparin. Thus, it is possible that the low level of plasma triglycerides found in deficient rats could at least partly be caused by an increased lipoprotein lipase activity in their tissues.

Boberg and Carlson (38) compared various lipid emulsions as substrates for lipoprotein lipase of human postheparin plasma. They found that chylomicrons and Intralipid had similar K_m values, which were lower than those of another synthetic emulsion, Ediol. In our experiments, using rat plasma, the K_m for VLDL from deficient rats was significantly lower than that of VLDL from normal rats, even though both lipoproteins at high concentrations underwent lipolysis at the same maximum rate. The K_m for Intralipid, with the same plasma, was higher than for either of the VLDL, but the maximum rate was again similar to that of the VLDL. Our results

show that to maintain the same submaximum rate of lipolysis the concentration of deficient VLDL required would be less than that of normal VLDL. Intralipid and perhaps chylomicrons would require a higher concentration. Hence, another factor in the explanation of the low level of VLDL in the plasma of deficient rats could be this difference between the values of K_m for VLDL from normal and deficient animals. The equilibrium concentration of VLDL would be lower in deficient rats because their VLDL undergoes lipolysis more readily at low concentration.

The main differences between VLDL from normal and deficient rats are in the fatty acid compositions of their lipids and in their ratios of protein to phospholipid. Hence, the observed difference in K_m between the two types of VLDL is further evidence of the influence which fatty acid composition has on binding between lipids and proteins (39). It is possible that this influence leads to the VLDL from deficient rats having a smaller average size than those of normal rats. Sarda and Desnuelle (40) have shown that the kinetics of hydrolysis of emulsified triglycerides by pancreatic lipase is dependent on substrate surface area rather than on total concentration. If VLDL from deficient rats were smaller in size, then their greater surface area per milligram of triglyceride could be responsible for their observed K_m being lower than that of VLDL from normal rats. However, the similarity of the ratios of hydrophobic core lipids to hydrophilic surface material in normal and deficient VLDL suggests that any difference in particle size between the two types of VLDL would be small. The difference in K_m could also be due to a stronger binding of triglyceride from deficient VLDL to the heparin lipase complex or to a more unstable protein-phospholipid surface coat in deficient VLDL that enables the lipid core to be more accessible to lipase action.

These results demonstrate again the importance of EFA in lipid-protein interactions. In the case of VLDL, we cannot as yet differentiate between the influence of linoleic acid on the properties of the triglyceride parts and of linoleic and arachidonic acid on the phospholipid parts of the lipoprotein.

As a result of the observations reported in this paper we can now explain the changes in the levels of liver and plasma lipid that are seen in rats maintained on a diet deficient in EFA. Due to a lack of prostaglandins, whose precursors are EFA (41, 42), deficient rats have an elevated level of FFA in their plasma (41, 43, 44). The resultant increase in input of FFA into the liver, by increasing the rate of production of triglyceride, raises the level of lipid in the liver. This effect is supplemented in rats fed a fat-free diet by an increased rate of synthesis of fatty acids by liver (4). The increasing level of triglyceride in liver stimulates production of VLDL until

after some weeks an equilibrium level of lipid is reached in the liver. This equilibrium level is higher than that found in normal rats. Unlike the fatty liver that occurs after administration of hepatic poisons, such as carbon tetrachloride, fatty livers in rats deficient in EFA do not continue to accumulate more fat but are in a steady state, where input of fat into the liver equals output (4). Thus, the flux of fat through the liver of deficient rats is greater than normal. The increased input from FFA is balanced by an increased rate of secretion of triglyceride into plasma (4). However, the level of lipid in the plasma does not rise, but is in fact lowered due to a combination of the increased affinity of lipoprotein lipase for VLDL in deficient animals and the increased amount of this enzyme in the tissues of these animals (this paper). Thus, the flux of triglyceride in the cycle from tissues via plasma FFA to liver and from liver via plasma VLDL back to tissues appears to be faster than normal in rats deficient in EFA.

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