Catabolism of chylomicron triacylglycerol and cholesteryl ester in genetically obese rats

T. G. Redgrave

Department of Physiology, University of Melbourne, Parkville, Victoria, 3052, Australia

Abstract The catabolism of chylomicrons was investigated in genetically obese rats and their nonobese littermates, and was compared with catabolism in older Sprague–Dawley rats with body weights similar to the obese rats and their younger controls. Labeled thoracic-duct lymph was collected from donor rats and the catabolism of the labeled chylomicrons was studied after a single intravenous injection or during steady intravenous infusion in unanesthetized, nonfasting, recipient rats. In the genetically obese rats clearances from the plasma of chylomicron triacylglycerol and cholesteryl ester were less than in their nonobese littermates. Fractional clearance rates were reduced for both triacylglycerol and cholesteryl ester but triacylglycerol turnover rate (mg min^{-1}) was greater than controls. Chylomicron triacylglycerol clearance was more efficient than cholesteryl ester clearance so that radioactivity remaining in the plasma was relatively depleted in triacylglycerol. The large-bodied old Sprague–Dawley rats showed no reduction in clearance of chylomicron radioactivity in comparison with younger controls.

These results suggest that hyperlipidemia in genetically obese rats may be due in part to an accumulation of chylomicron remnants in the plasma. Flotation characteristics of plasma lipoproteins in the obese rats were consistent with this interpretation. However, separate experiments showed that genetically obese, fasting rats also accumulated more triacylglycerol in the plasma after injection of Triton WR 1339. The enlarged plasma triacylglycerol pool appears to derive from a mixture of hepatic and intestinal triacylglycerol-rich lipoproteins which, together, overload their common removal mechanism. Addition of cholesterol to the diets of the obese rats exacerbated their hyperlipemia and hepatic steatosis whereas their nonobese littermates and the large-bodied Sprague–Dawley rats were unaffected.

Supplementary key words Zucker rats · fractional catabolic rate · turnover

In addition to being obese, the genetically obese Zucker rat is hyperlipemic (1, 2). Plasma concentrations of triacylglycerol and cholesterol are both increased but most of the increase in plasma lipids is accounted for by plasma triacylglycerol (3). Recent studies by Schonfeld, Felski, and Howald (3) have shown that plasma VLDL is increased several-fold but LDL and HDL are also moderately increased. It was considered by these workers that the hyperlipemia of genetically obese Zucker rats was due to an increased hepatic production of VLDL and they reported that the activity of lipoprotein lipase in adipose tissue exceeded that of controls. There appears to have been no previous study of clearance from the plasma of hepatic VLDL or intestinal lymph lipoproteins. In this study clearance of intestinal lymph lipoproteins (predominantly chylomicrons) has been studied. At the same time studies have been made of chylomicron metabolism in old Sprague–Dawley rats of body weights similar to obese Zucker rats. Both old and young Sprague–Dawley rats have normal blood lipid levels.

METHODS

Animals

Genetically obese (Zucker) rats and their nonobese littermates were generously provided by Professor George Singer of La Trobe University, Bundoora, Victoria, 3083. Three experiments using Zucker rats were performed.

Experiment 1 was a study of chylomicron catabolism after a single injection of chylomicrons in rats fed a standard laboratory ration. For comparison obese and nonobese Zucker rats and Sprague–Dawley rats were studied.

Experiment 2 was a study of chylomicron catabolism in Zucker rats using a steady state infusion technique that was carried out to confirm and extend the observations made in experiment 1. These rats were fed the usual ration prior to the experiment, but received evaporated milk during studies of chylomicron catabolism.

Experiment 3 was a study of the effects of added dietary cholesterol on the obese rats in comparison

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FFA, free fatty acid.

with their nonobese littermates and Sprague–Dawley rats. For experiment 1, the rats were 14–16 weeks old; for experiment 2, 18–26 weeks old, and for experiment 3, 10–14 weeks old. Sprague–Dawley rats were obtained from Hawthorn Park Research Laboratories, Mittagong, NSW at about 200 g body weight. Some were maintained on commercial rat pellets (GR2+, Clark King Co., Melbourne) until they were about 10 months of age, when they weighed 400–450 g.

A 1% cholesterol diet was prepared by mixing a solution of cholesterol in ethyl ether with standard rat pellets and then evaporating the ether. In experiment 3, Zucker rats were fed the diet for 14 days and Sprague–Dawley rats were fed the diet for 24 days.

Collection of radioactive chylomicrons

a) Single injection studies (experiment 1). Sprague–Dawley rats weighing about 200 g were prepared with a thoracic duct cannula and a gastric cannula (4). Details of preparation and management have been given previously (5). Fatty lymph was collected after a solution of 1% cholesterol in 0.3 ml of corn oil had been administered via the gastric cannula. The test meal contained 10 μCi of [4-^{14}C]cholesterol and 5 μCi of [2-^{3}H]glyceryl trioleate (both obtained from the Radiochemical Centre, Amersham, U. K.). Lymph was collected only when it was clearly milky. It was collected and allowed to clot at 20 ± 3°C, and was used within 48 hr of collection after filtering through several layers of gauze. Fatty lymph was diluted with nonfatty lymph from fasting rats to a final triacylglycerol concentration of 7–10 mg ml^{-1}.

b) Steady infusion studies (experiment 2). Nonobese rats of the Zucker strain were prepared with a thoracic duct cannula and a cannula inserted through the stomach wall and passed into the upper small intestine so the tip lay 2–3 cm beyond the pylorus. After surgery diluted evaporated milk (triacylglycerol concentration 23 mg ml^{-1}) was infused steadily overnight at 1.2 ml hr^{-1} via the intestinal cannula. The next morning the infusion was replaced with a test meal prepared in the following way. To 30 μCi of 1α, 2α(n)[^{3}H]cholesterol and 10 μCi of [1-^{14}C]palmitic acid (Radiochemical Centre, Amersham, U. K.) was added 0.1 ml of 0.1 M KOH in 50% ethanol to convert the fatty acid to its potassium salt, and then 1 ml of the diluted evaporated milk was added rapidly. This mixture was then infused over 50–60 min into the intestinal cannula. Lymph was collected for 4 hr at 20 ± 2° into a vessel containing EDTA and ethyl mercurithiosalicylate (final concentrations 2.7 and 0.25 mM, respectively).

Chylomicrons ($S_f > 400$) were isolated from lymph in a superspeed 65 ultracentrifuge (Measuring and Scientific Equipment Ltd., London S.W. 1, U. K.) using a discontinuous density gradient in a swinging bucket rotor (6). The washed chylomicrons were suspended in 0.15 M NaCl to final concentrations of 10–15 mg. ml^{-1}. Chylomicrons prepared in this way contained about 0.1 μCi. ml^{-1} each of [^{3}H]cholesteryl ester and [^{14}C]triacylglycerol. Cholesteryl ester accounted for 60–75% of ^{3}H and triacylglycerol accounted for 85–90% of ^{14}C.

Chylomicron catabolism

a) Single injection studies (experiment 1). One ml of radioactive lymph was injected into the tail vein of each recipient rat without anesthesia and exactly 10 min later, under ether anesthesia, a blood sample was taken by cardiac puncture and the liver was removed. Details have been given previously (5). Experiments were carried out between 10.00 and 14.00 hr on nonfasting rats.

b) Steady infusion studies (experiment 2). One or two days before the study, obese and nonobese rats were prepared with a jugular venous cannula and a carotid arterial cannula. The venous cannula was 0.5 mm ID × 0.9 mm OD vinyl tubing and the arterial cannula was 0.4 mm ID × 0.8 mm OD vinyl tubing (Dural Plastics & Engineering Pty Ltd, Dural N. S. W. 2158). The arterial cannula was inserted for 3 cm so the tip was lying free in the aortic blood stream. Heparin was assiduously avoided. To avoid interference with thoracic duct lymph drainage, both cannulations were performed on the right side. Surgery was performed under ethyl ether anesthesia and postoperatively the rats were placed in Bollman restraining cages (7). The cannulae were brought out through the skin at the back of the neck; they were filled with 0.15 M NaCl and tied in a knot at their distal ends to prevent entrance of blood. Animals were given evaporated milk to drink overnight and studies of chylomicron catabolism were performed the next morning. Fat absorption was checked after the completion of the study by examining intestinal content and lacteals. Only absorptive animals were used for these studies.

Radioactive chylomicrons were infused steadily via the venous cannula at 0.04 ml min^{-1} for 30 min. A steady state is reached in this time (8, 9). At 25 and 30 min, blood samples were taken via the arterial cannula. All samples were 0.4 ml and were placed into microcentrifuge tubes containing 5 units of heparin. Plasma was separated by centrifugation at 800 g for 15 min.

Plasma volume

In six obese Zucker rats and six nonobese rats a suspension of ^{51}Cr-labeled red blood cells (10) was injected into a tail vein and a blood sample was taken 10 min later. The hematocrit was determined (allowing for plasma trapping by cells) and radioactivity was counted with a Nuclear Chicago Auto-Gamma Counter 1185. Plasma volume was then calculated from the red cell mass and the hematocrit value. Plasma volume was 2.8 ± 0.11 ml/100 g body weight for obese Zucker rats, and 4.1 ± 0.08 ml/100 g body weight for nonobese rats. For the large-bodied Sprague–Dawley rats, plasma volume was calculated from the relationship given by Wang and Hegsted (11).

Triton WR 1339 studies

Under ether anesthesia, obese rats and their nonobese littermates were prepared with a cannula in the right carotid artery as before. They were then placed in restraining cages and given tap water to drink but nothing to eat for 20 hr. A blood sample was taken via the arterial cannula and 1 ml of 10% Triton WR 1339 (Sterling Pharmaceuticals Pty Ltd, Sydney, N. S. W.) in 0.15 M NaCl was given through the cannula. Five additional blood samples of 0.5 ml were taken at intervals over the next 90 min. Samples were placed into tubes containing 10 U of heparin and centrifuged at 800 *g* for 15 min; then 0.2 ml of plasma was extracted for determination of triacylglycerol concentrations.

Analytical

Lipids were extracted from liver, plasma, and lymph by the method of Folch, Lees, and Sloane Stanley (12). For the single injection studies, after purification of the extracts, aliquots were applied to silicic acid columns for separation of cholesteryl esters from free cholesterol and triacylglycerols. All separations were checked by thin-layer chromatography. Portions of the eluates were taken for determination of radioactivity with a Beckman LS250 liquid scintillation system by evaporating off the eluting solvents and dissolving the sample in a solution of 0.5% PPO in toluene. Quenching was corrected in conjunction with the automatic quenching correction facility available in this system by external standardization from curves prepared with [^{14}C]toluene and [^{3}H]toluene. Under double-isotope conditions, the mean counting efficiency was 66% for ^{14}C and 39% for ^{3}H.

For the steady infusion studies, the lipid extracted from 0.1 ml of plasma was dissolved in 0.25 ml of chloroform–methanol 2:1 (v/v) and duplicate 0.1 ml portions were applied to 0.25 mm layers of silica gel G. After chromatography in the solvent mixture light petroleum (bp 60–80°C)–ethyl ether–acetic acid 80:20:1, the bands corresponding to triacylglycerol and cholesteryl ester were scraped into scintillation vials. The fluorescent dye Blankophor BHC 766 (Bayer, Leverkusen) was used to detect the bands under ultraviolet light. Counting of radioactivity was as before, after eluting the lipids with 10 ml of 0.5% PPO in toluene.

Cholesterol was measured after saponification by the method of Zlatkis and Zak (13), triacylglycerols by the method of Van Handel and Zilversmit (14), and lipid phosphorus on digested samples by the method of Eibl and Lands (15). The distribution of cholesterol in rat lipoproteins was measured after separation in a discontinuous gradient in a single 24 hr centrifugation at 284,000 *g* (max) as described previously (16).

RESULTS

At 14–16 weeks of age the obese female Zucker rats used in experiment 1 were considerably heavier than their nonobese littermates, as shown in **Table 1**, and in comparison they had greater concentrations of cholesterol and triacylglycerol in the plasma. The female Sprague–Dawley rats of experiment 1 were much older than the Zucker rats, but their body weights were comparable with the obese Zucker rats. Plasma concentrations of cholesterol and triacylglycerol in the Sprague–Dawley rats were comparable with those in the obese Zucker rats. Liver weights varied in proportion to body weights and liver cholesterol concentration was similar in all groups. Liver triacylglycerol was clearly increased in obese Zucker rats compared with their littermates.

According to previous experiments indicating the role of cholesteryl ester-enriched remnant particles in the metabolism of chylomicrons, after 10 min in normal rats most injected lymph cholesteryl ester was found in the liver but only a relatively small proportion of injected lymph triacylglycerol was found there. **Table 2** shows that nonobese Zucker rats also conformed to this pattern. Chylomicron triacylglycerol was rapidly removed from the plasma but only 11% of the dose was recovered in the liver. Chylomicron cholesteryl ester was removed from the plasma somewhat more slowly, but the majority of the material cleared from the plasma was recovered in the liver. For the obese Zucker rats, chylomicron triacylglycerol was removed from the plasma less efficiently than for the nonobese rats, and clearance

TABLE 1. Body weight, plasma glucose, and plasma and hepatic lipids of Zucker rats and Sprague–Dawley rats of experiment 1[a]

	Zucker Rats		Sprague–Dawley Rats	
	Obese Females (3)	Nonobese Females (3)	Old Females (4)	Young Females (4)
Body weight (g)	304 ± 12.4[b]	175 ± 2.5	379 ± 16.6	221 ± 5.0
Plasma cholesterol (mg/dl)	73 ± 2.8[b]	44 ± 2.0	84 ± 5.1	82 ± 7.5
Plasma triacylglycerol (mg/dl)	194 ± 15.3[b]	31 ± 12.1	137 ± 21.6	107 ± 23.0
Plasma glucose (mg/dl)	192 ± 8.5	161 ± 8.6	131 ± 21.4	192 ± 6.2
Liver weight (g)	9.8 ± 0.59[c]	6.9 ± 0.18	14.4 ± 0.95	9.6 ± 0.27
Liver cholesterol: concn. (mg/g)	2.1 ± 0.08	2.1 ± 0.07	2.8 ± 0.31	1.9 ± 0.2
Liver triacylglycerol: concn. (mg/g)	11.8 ± 2.68[c]	2.6 ± 0.04		6.5 ± 1.0

[a] Results are means ± SEM; the numbers of animals are shown in parentheses.
[b] $P < 0.001$, by Student's t test comparing the obese rats with controls.
[c] $P < 0.01$.

of chylomicron cholesteryl ester was substantially reduced. Recovery in the liver of the material that had left the plasma still showed a clear partition of triacylglycerol from cholesteryl ester. In the old large-bodied female Sprague–Dawley rats clearance from the plasma was also slower than in the nonobese Zucker rats, but was similar to the young female Sprague–Dawley rats. Again there was a separation of constituents recovered in the liver.

The above results suggested that female obese Zucker rats might have a reduced capacity to clear absorbed dietary cholesterol from their plasma. **Table 3** shows that, compared with the injected chylomicrons, after 10 min residual radioactivity in the plasma contained less triacylglycerol than cholesteryl ester in all groups. The relative depletion of chylomicron triacylglycerol was greater in the Zucker rats than in the Sprague–Dawley rats, and greater in the nonobese Zucker rats than in the obese. In all groups radioactivity recovered in the liver was consistently depleted in triacylglycerol radioactivity by about 80% compared with the injected radioactivity in lymph.

After steady infusion for 30 min, plateau concentrations of cholesteryl ester and triacylglycerol had been reached, and samples taken at 25 min were 98.0 ± 4.18% (eight observations) of those taken at 30 min.

After steady infusion for 30 min, the plasma con-

TABLE 2. Catabolism of injected chylomicron triacylglycerol and cholesteryl ester in experiment 1[a]

	Zucker Rats		Sprague–Dawley Rats	
	Obese Females	Nonobese Females	Old Females	Young Females
Clearance from plasma (% of injected dose)				
Triacylglycerol	85 ± 3.3[b]	99 ± 0.2	81 ± 5.0	77 ± 6.8
Cholesteryl ester	42 ± 8.5[b]	82 ± 3.9	58 ± 5.1	56 ± 2.4
Recovery in liver (% of injected dose)				
Triacylglycerol	8 ± 1.3	11 ± 1.5	11 ± 0.8	12 ± 2.1
Cholesteryl ester	42 ± 8.5	76 ± 11.0	55 ± 9.9	46 ± 6.0
(% of cleared dose)				
Triacylglycerol	9 ± 1.2	11 ± 1.5	13 ± 0.5	17 ± 4.2
Cholesteryl ester	100 ± 0.4	92 ± 9.6	92 ± 10.1	86 ± 11.4

[a] Rats were given a single injection of fatty lymph labeled with [^{14}C]cholesterol and [^{3}H]glyceryl trioleate and the percentage of injected label cleared from the plasma and appearing in the liver was measured after 10 min. Results are means ± SEM, numbers of animals as in Table 1.
[b] Significantly different from nonobese controls $P < 0.02$ by Student's t test.

TABLE 3. Ratio of radioactive triacylglycerol: cholesteryl ester in plasma and liver in experiment 1[a]

	Zucker Rats		Sprague–Dawley Rats	
	Obese Females	Nonobese Females	Old Females	Young Females
Plasma	0.24 ± 0.03	0.05 ± 0.02	0.44 ± 0.08	0.50 ± 0.07
Liver	0.19 ± 0.01	0.15 ± 0.01	0.21 ± 0.03	0.26 ± 0.04

[a] The ratio of residual radioactivity in plasma and liver 10 min after injection is compared to the ratio of radioactive triacylglycerol: cholesteryl ester in the injected lymph = 1.00. Results are means ± SEM.

tent of infused radioactivity was greater in the obese Zucker rats than in nonobese controls (**Table 4**). Confirming the findings after a single injection in experiment 1, after a steady infusion cholesteryl ester was cleared from the plasma less efficiently than triacylglycerol but clearance of both was reduced. As described by Harris and Felts (9), the fractional catabolic rate can be calculated in the steady state if it is assumed that the volume of distribution of chylomicrons is approximated by the plasma volume. As shown in Table 4, the fractional catabolic rate calculated in this way is significantly less in obese than in nonobese rats for both triacylglycerol and cholesteryl ester. The half-life calculated directly from the fractional catabolic rate (ln 2 ÷ FCR) was correspondingly greater in the obese rats.

The pool of plasma triacylglycerol is not metabolically homogeneous but it is not known what fraction of this pool is represented by the infused chylomicron triacylglycerol. If it is assumed that the pool is homogeneous, then the turnover (mg·min^{-1}) can be calculated from the plasma triacylglycerol concentration, the plasma volume, and the fractional catabolic rate. The results of this calculation are also shown in Table 4. Turnover of plasma triacylglycerol is greater in the obese rats. Similar calculations for plasma cholesteryl ester were not attempted because the pool cannot be defined.

Harris and Felts (9) also calculated the fractional catabolic rates from the disappearance curves plotted on semilog coordinates. This was attempted in the present study but the results were unsatisfactory because the disappearance was not first-order over the 10 min period and, also, the actual counts were too low for accurate measurement after 5 min because of the observed very rapid fractional catabolic rates (Table 4). Hence more extensive kinetic analysis was not possible with this experimental design. The fractional catabolic rate of chylomicron triacylglycerol for control nonobese rats was much faster than that reported by Harris and Felts (9). The difference between the fractional catabolic rates for triacylglycerol and cholesterol ester in control nonobese rats was statistically significant by the paired t test ($P < 0.025$).

Fig. 1 shows the accumulation of triacylglycerol in the plasma of fasted obese and nonobese rats after intravascular injection of Triton WR 1339, which is known to prevent clearance of triacylglycerol-rich lipoproteins (17). It is clear from Fig. 1 that the obese rats accumulate triacylglycerol in the plasma much more rapidly than do the nonobese controls. The mean accumulation rates were 134 ± 15.3 μg.·min^{-1} for nonobese rats and 349 ± 11.9 μg·min^{-1} for obese rats.

In experiment 3, 1% cholesterol was added to the

TABLE 4. Catabolism of steadily infused chylomicron triacylglycerol and cholesteryl ester in experiment 2[a]

	Obese Zucker Rats		Nonobese Zucker Rats	
	Triacylglycerol	Cholesteryl Ester	Triacylglycerol	Cholesteryl Ester
Steady state content in plasma compartment (% of injected dose)	10.6 ± 1.86[b]	27.3 ± 5.71	3.6 ± 0.66	14.8 ± 4.06
Fractional catabolic rate (min^{-1})	0.43 ± 0.11[c]	0.20 ± 0.07[c]	1.35 ± 0.33	0.50 ± 0.10
Catabolic half-life (min)	3.2 ± 0.39[d]	5.7 ± 1.19	0.81 ± 0.13	3.1 ± 0.84
Turnover (mg·min^{-1})	5.1 ± 1.2[e]		2.0 ± 0.33	
Plasma specific activity (fraction of specific activity of injected chylomicrons)	0.17 ± 0.05[f]		0.37 ± 0.08	

[a] Rats were given, for 30 min, a steady intravenous infusion of chylomicrons labeled with [^{3}H]cholesterol and [^{14}C]triacylglycerol. Samples of plasma taken at 30 min were assayed for radioactivity to calculate the fractional catabolic rate and $t_{\frac{1}{2}}$. Results are means ± SEM from 7 obese and 12 nonobese rats.

[b] Significantly different from nonfatty controls, $P < 0.005$ by Student's t test.

[c] $P < 0.025$.

[d] $P < 0.001$.

[e] Calculated assuming that plasma triacylglycerol forms a homogeneous pool. The observed difference is statistically significant, $P < 0.025$ by Student's t test.

[f] $P < 0.05$ by Student's t test.

TABLE 5. Plasma and liver lipids of cholesterol-fed rats in experiment 3[a]

	Zucker Rats				Sprague–Dawley Rats (Male)	
	Obese		Nonobese			
	Male (3)	Female (6)	Male (9)	Female (4)	Cholesterol-fed (5)	Normal-fed (6)
Body weight (g)[b]	327 ± 17.1	272 ± 8.6	201 ± 5.1	157 ± 3.6	463 ± 9.5	449 ± 10.3
Plasma cholesterol[b] (mg/dl)	122 ± 16.6	100 ± 7.2	57 ± 2.7	54 ± 1.3	66 ± 3.6	78 ± 5.8
Plasma triacylglycerol[b] (mg/dl)	181 ± 37.2	272 ± 39.1	16 ± 3.2	27 ± 10.7	81 ± 6.9	116 ± 22.5
Liver weight (g)[b]	15.7 ± 2.53	13.2 ± 0.86	7.7 ± 0.35	6.9 ± 0.24	15.7 ± 0.68	14.5 ± 0.54
Liver lipids: cholesterol (mg/g)	5.0 ± 0.97	5.2 ± 0.82	4.3 ± 0.28	3.0 ± 0.10	6.6 ± 1.23	1.9 ± 0.06
Triacylglycerol (mg/g)[c]	44.5 ± 12.25	105.3 ± 28.9	7.0 ± 0.45	8.5 ± 0.45	10.7 ± 0.65	6.7 ± 0.96
Phospholipid (mg/g)	25.9 ± 0.09	24.5 ± 1.14	26.8 ± 1.35	21.8 ± 5.13	31.2 ± 0.95	32.6 ± 0.77
Total lipids (mg/g)[c]	75.3 ± 14.00	135.1 ± 28.5	38.1 ± 1.73	33.2 ± 5.62	48.5 ± 1.38	41.2 ± 1.50

[a] Results are means ± SEM; the numbers of animals are shown in parentheses. All animals were fed standard rat pellets plus 1% cholesterol.
[b] $P < 0.001$ by analysis of variance.
[c] $P < 0.01$.

diet of Zucker rats and, as shown in **Table 5**, there was a further increase in the hyperlipemia of the obese females, of about 37% for cholesterol and 40% for triacylglycerol (cf. Table 1). In contrast the nonobese Zucker rats and the Sprague–Dawley rats showed no increases in either plasma cholesterol or triacylglycerol when fed cholesterol. An unexpected finding in the cholesterol-fed obese Zucker rats was the massive increase in hepatic triacylglycerol (Table 5). This did not occur when the nonobese Zucker rats or the Sprague–Dawley rats were fed cholesterol. In the female obese Zucker rats, cholesterol feeding resulted in a total lipid content of the liver of 13% and the liver was visibly whitened.

The plasma lipoproteins from the Zucker rats were separated according to their densities and **Table 6** shows that, compared with their nonobese littermates, the obese Zucker rats had a fourfold increase in plasma VLDL cholesterol and also had smaller increases in LDL and HDL cholesterol. The relative contribution of VLDL to total plasma cholesterol increased from 8% in the controls to 18% in the obese Zucker rats.

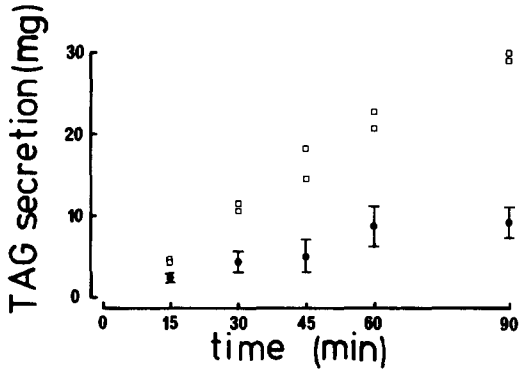

Fig. 1. Secretion of triacylglycerol into the plasma compartment of fasted obese (□) and nonobese (●) Zucker rats. After injection of 100 mg of Triton WR 1339, secretion of triacylglycerol was calculated from the product of the increment in plasma triacylglycerol concentration and plasma volume, over the period of 90 min.

DISCUSSION

Apart from their marked obesity, fatty Zucker rats are well known to show a number of other abnormalities in lipid metabolism. Their hyperlipemia is well-described, plasma FFA concentration is elevated and FFA mobilization from adipose tissue is increased (18). It is known that most of the increase in plasma lipids is accounted for by lipoproteins of density $<1.006 g \cdot ml^{-1}$ (3) but the mechanism of this increase has not been elucidated. It has been suggested that clearance of plasma lipoproteins is impaired but there is no direct evidence to support this view. Measurements of adipose tissue lipoprotein lipase have shown either no change (19) or an increased enzyme activity (3).

The present experiments provide direct support for the suggestion that clearance of primary lipoproteins is overloaded. Primary triacylglycerol-rich lipoproteins produced by the liver (VLDL) or the intestine (predominantly chylomicrons) share a common mechanism of clearance (20) whereby most of the triacylglycerol is first removed in peripheral tissues by the action of lipoprotein lipase. The cholesterol-enriched intermediate particle or chylomicron remnant is then separately removed by the liver (5, 21,

TABLE 6. Distribution of plasma cholesterol in separated lipoproteins of Zucker rats[a]

Lipoprotein Density	Cholesterol Content		Relative Distribution	
	Obese	Nonobese	Obese	Nonobese
g/ml	*mg/dl*	*mg/dl*	%	%
<1.006	19.1 ± 2.87[b]	4.8 ± 1.76	18.1 ± 2.7[c]	7.8 ± 2.5
1.006–1.019	6.3 ± 1.58	6.7 ± 1.37	5.8 ± 1.5[c]	11.2 ± 1.8
1.019–1.063	24.2 ± 3.90	14.5 ± 1.81	21.7 ± 2.5	24.7 ± 2.7
1.063–1.21	55.3 ± 4.38[b]	29.4 ± 1.65	52.1 ± 3.4	50.8 ± 3.4
>1.21	2.6 ± 0.53	3.2 ± 0.29	2.3 ± 0.3[d]	5.5 ± 0.5

[a] Plasma lipoproteins were separated by density gradient ultracentrifugation (16). Results are means ± SEM; nine obese and five nonobese rats were studied.
[b] $P < 0.01$, comparison by Student's t test obese vs. nonobese.
[c] $P < 0.05$.
[d] $P < 0.001$.

22). In the obese Zucker rats clearances of both triacylglycerol and cholesterol from the plasma were delayed, but recoveries in the liver indicated that the removal process was qualitatively similar to the mechanism in control rats. The impairment was more marked for cholesterol than for triacylglycerol. In the old Sprague–Dawley rats of body weight similar to the obese Zucker rats, there was no impairment in clearance from the plasma compared with values for Sprague–Dawley rats of lesser body weight.

Two experimental techniques have been used to study chylomicron catabolism in these experiments. The results showing delayed catabolism after a single intravenous injection have been confirmed and extended by studying kinetic parameters of clearance in unanesthetized rats during steady infusion of chylomicrons. In all experiments particular care was taken to ensure that the animals were actively absorbing fat from their dietary intake so that the injected radioactivity would act as a tracer of normal physiological clearance. In the steady state, rates of ingress and egress of radioactivity from the plasma compartment are equal, so the fractional catabolic rate must vary inversely with the steady state content of radioactivity in the plasma. This is supported by the data of Table 4, but it is clear that triacylglycerol and cholesteryl ester are catabolized differently within each group (by the paired t test, the steady state content of cholesteryl ester is significantly different from triacylglycerol for each group, $P < 0.025$). This difference presumably arises because the catabolism of chylomicrons is a two-step process, viz.

(*i*) chylomicrons → remnants

(*ii*) remnants → removal by the liver

Hence the measured clearance of chylomicron triacylglycerol is a composite of the two processes involved and the fractional clearance of chylomicron cholesteryl ester cannot be faster than that of chylomicron triacylglycerol. Furthermore, such a complex clearance mechanism cannot be described by a first-order rate equation, but it may be described by the sum of two exponentials as was recently shown for chylomicron catabolism in rabbits (23). It should also be noted that the volume of distribution of chylomicrons was larger than the plasma volume in rabbits (23); if this is the case in rats it will invalidate the assumptions used in calculating the fractional clearance rates in the steady state in the present experiments and in those of Harris and Felts (9).

Calculations of turnover ($mg \cdot min^{-1}$) require definition of the mass that is being traced with the infused radioactivity. However, present techniques do not allow chylomicrons and their catabolic products to be separated satisfactorily from hepatic VLDL and their catabolic products, so the results given for turnover in Table 4 are at best an approximation and may be misleading. Nevertheless it should be stressed that, in the steady state, rates of ingress and egress of triacylglycerol and cholesteryl ester mass are equal. A difference in turnover therefore implies a difference in input into the plasma pool. The results after injection of Triton WR 1339 indicate that there is a difference in input in the fasting state, which can be interpreted as increased hepatic production of VLDL in the obese rat (24). However, the rates of input in the fasting state (134 and 349 $\mu g \cdot min^{-1}$ for control and obese rats, respectively) are much less than the turnovers of 2.0 and 5.1 $mg \cdot min^{-1}$ in the fed state. This inconsistency will in part be accounted for by input of

chylomicrons from the intestine after feeding, but it might also be an indication of metabolic nonhomogeneity of the plasma triacylglycerol pool.

The obese Zucker rats showed moderate hyperlipemia more marked for plasma triacylglycerol than for cholesterol, and more marked in the female obese rats than in the males (Table 5). At the same time the obese rats, especially the females, showed an accumulation of triacylglycerol in the liver that was considerably aggravated by adding cholesterol to the diet. Fillios et al. (25, 26) reported that feeding extra cholesterol and cholic acid produced a fatty liver in the obese Zucker rat. The mechanism of production of this hepatic steatosis is open to interpretation.

The present observations indicating defective catabolism of chylomicrons in the plasma compartment may be related to further abnormalities during catabolism of the chylomicron remnant by the liver. Additional dietary cholesterol will be transported as chylomicron remnants to the liver and will impose a further load on the hepatic catabolic processes. However further experiments will be required to examine the role of chylomicrons and their remnants in the development of hepatic steatosis.

In the obese Zucker rat the predominant increase in plasma lipoproteins was found to be of density $<1.006 g \cdot ml^{-1}$ in these experiments, confirming the recent observations of Schonfeld et al. (3). Chylomicron remnants still contain about 80% triacylglycerol (21) so failure to clear remnants adequately will lead to hypertriglyceridemia in addition to hypercholesterolemia, with the predominant increase in plasma lipids at a density $<1.006 g \cdot ml^{-1}$.

These studies have shown that injected chylomicrons are depleted of their triacylglycerol content reasonably efficiently in obese Zucker rats compared with Sprague–Dawley rats. This observation is consistent with the reports of normal or increased lipoprotein lipase activity in obese Zucker rats (3, 19). However, chylomicron cholesteryl ester is cleared from the plasma significantly less efficiently, suggesting that chylomicron remnants contribute to hyperlipidemia in the obese Zucker rat. This evidence does not exclude a contribution to the hyperlipidemia from hepatic VLDL, which shares a common removal mechanism with chylomicrons (20).

The results also show that overall turnover of plasma triacylglycerol is not necessarily impaired in association with defective plasma clearance of chylomicron remnants. If the initial phase in the catabolism of chylomicrons to remnants is unimpaired, a study of triacylglycerol removal alone may appear normal because 80% or more of chylomicron triacylglycerol content is removed during the process of conversion of chylomicrons to remnants. However, a defect in the subsequent clearance of chylomicron remnants will be revealed by studying simultaneously the clearance of chylomicron cholesterol ester. Such a defect will result in hypertriglyceridemia due to the residual triacylglycerol content of the chylomicron remnants.

This work was supported by a grant from the National Health and Medical Research Council. Elaine Softley gave skillful technical assistance.

Manuscript received 23 November 1976 and accepted 26 April 1977.

REFERENCES

1. 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.
2. Barry, W. S., and C. A. Bray. 1969. Plasma triglycerides in genetically obese rats. *Metabolism.* **18:** 833–839.
3. 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.
4. Bollman, J. L., J. C. Cain, and J. H. Grindlay. 1948. Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. *J. Lab. Clin. Med.* **33:** 1349–1359.
5. Redgrave, T. G. 1973. Cholesterol feeding alters the metabolism of thoracic-duct lymph lipoprotein cholesterol in rabbits but not in rats. *Biochem. J.* **136:** 109–113.
6. Minari, O., and D. B. Zilversmit. 1964. Behavior of dog lymph chylomicron lipid constituents during incubation with serum. *J. Lipid Res.* **4:** 424–436.
7. Bollman, J. L. 1948. A cage which limits the activity of rats. *J. Lab. Clin. Med.* **33:** 1348.
8. Bishop, D. M. 1972. Removal of chylomicron triglyceride from the bloodstream. Doctoral thesis, Australian National University, Canberra.
9. Harris, K. L., and J. M. Felts. 1970. Kinetics of chylomicron triglyceride removal from plasma in rats: a comparison of the anesthetized and the unanesthetized states. *J. Lipid Res.* **11:** 75–81.
10. Lajtha, L. G. 1961. The Use of Isotopes in Haematology. Blackwell, Oxford, U.K. 8–16.
11. Wang, C. F., and D. M. Hegsted. 1949. Normal blood volume, plasma volume and thiocyanate space in rats and their relation to body weight. *Amer. J. Physiol.* **156:** 218–226.
12. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
13. Zlatkis, A., and B. Zak. 1969. Study of a new cholesterol reagent. *Anal. Biochem.* **29:** 143–148.
14. Van Handel, E., and D. B. Zilversmit. 1957. Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* **50:** 152–157.
15. Eibl, H., and W. E. M. Lands. 1969. A new, sensitive determination of phosphate. *Anal. Biochem.* **30:** 51–57.

16. Redgrave, T. G., D. C. K. Roberts, and C. E. West. 1975. Separation of plasma lipoproteins by density-gradient ultra-centrifugation. *Anal. Biochem.* **65:** 42–49.
17. Otway, S., and D. S. Robinson. 1967. The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Physiol. (London)* **190:** 321–332.
18. Zucker, L. M., and H. N. Antoniades. 1972. Insulin and obesity in the Zucker genetically obese rat "Fatty". *Endocrinology.* **90:** 1320–1330.
19. Bray, G. A., W. S. Barry, and S. Mothon. 1970. Lipogenesis in adipose tissue from genetically obese rats. *Metabolism.* **19:** 839–848.
20. Brunzel, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
21. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.
22. Mjøs, O. D., O. Faergeman, R. L. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and in intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
23. Redgrave, T. G., K. B. Dunne, D. C. K. Roberts, and C. E. West. 1976. Chylomicron metabolism in rabbits fed diets with or without added cholesterol. *Atherosclerosis.* **24:** 501–508.
24. Schonfeld, G., and B. Pfleger. 1971. Over production of very low density lipoprotein by livers of genetically obese rats. *Amer. J. Physiol.* **220:** 1178–1181.
25. Fillios, L. C., and S. Saito. 1965. Hepatic protein synthesis and lipid metabolism in genetically obese rats. *Metabolism.* **14:** 734–745.
26. Fillios, L. C., and O. Yokono. 1966. Liver RNA polymerase activity in genetically obese and lean rats fed cholesterol. *Metabolism.* **15:** 279–285.