Plasma Lipid Secretion and Clearance in Hyperlipidemic JCR:LA-Corpulent Rats

James C. Russell, Dorothy G. Koeslag, Roger M. Amy, and Peter J. Dolphin

The JCR:LA-corpulent rat is an obese, hyperlipidemic, hyperinsulinemic strain that is atherosclerosis-prone and develops myocardial lesions. The hyperlipidemia is due to elevated plasma levels of a large relatively triglyceride-rich very low density lipoprotein (VLDL). Both corpulent and lean male and female rats were studied. Postheparin lipid clearance and apparent hepatic secretion rate after Triton WR1339 inhibition of lipoprotein lipase were determined. The concentrations of cholesterol and cholesteryl esters were not significantly altered by either treatment. Triglycerides showed rapid postheparin clearance in corpulent rats. The apparent hepatic secretion rate was markedly higher in corpulent male rats than in lean male rats, and the rate in corpulent females was again higher, reflecting the higher serum triglyceride concentrations in corpulent female rats. The relative secretion rate of C:48 triglyceride molecular species was lower than that of the C:50 to C:56 species, while the postheparin clearance of C:48 triglyceride molecular species was impaired compared to the C:50 species and those with higher carbon numbers. This effect was more marked in the male than in the female corpulent rats. The results indicate that VLDL hyperlipidemia in the corpulent rat is due to hepatic hypersecretion of VLDL and not to a defect in lipoprotein lipase. Further, the atherogenesis that is characteristic of the corpulent male rat may be related to the differential metabolism of fatty acids. (Arteriosclerosis 9:869–876, November/December 1989)

The JCR:LA-corpulent rat incorporates the corpulent (cp) gene isolated by Koletsky.1,2 The original strain was derived from a cross of the spontaneously hypertensive (SHR) and Sprague-Dawley rat strains and was hypertensive, hyperlipidemic, and prone to a fulminant atherosclerosis. Hansen3 incorporated the cp gene into two inbred strains, the SHR/N and the LA/N. Repeated backcrosses (more than 12 times) were made to the parent strains to give two congenic strains. Rats that are homozygous for the cp gene (cp/cp) are obese, while rats that are heterozygous (cp/+) or homozygous normal (+/+) are lean and indistinguishable from the parent strain. The LA/N-cp strain has been described by Elwood et al.4 and studied as a model for obesity and the effects of high sugar intake. The SHR/N-cp rat is highly sensitive to dietary sucrose and is regarded as a model for the study of obesity and insulin resistance.5

In the course of the development of the LA/N-cp congenic strain, breeding stock from the fifth backcross was used to establish a colony in our laboratories. This colony differs from the fully congenic LA/N-cp and has recently been designated JCR:LA-cp. We have described this strain in previous publications.6,7 The cp/cp male rats spontaneously develop both atherosclerotic and myocardial lesions, while cp/cp female and lean rats are spared.7 The cp/cp rats have abnormal insulin and glucose metabolism that is manifested in an extreme and age-dependent hyperplasia of the insulin secreting pancreatic B cells.8 This is accompanied by an insulin resistance with very high circulating insulin levels and impaired glucose tolerance.9 These abnormalities are more marked in the cp/cp male than in the cp/cp female animals, which resemble the fatty Zucker rat, another obese strain of rat that does not develop cardiovascular disease.10

The cp/cp rats also exhibit a marked hyperlipidemia.11 This is due to greatly elevated levels of a triglyceride-rich very low density lipoprotein (VLDL) leading to a hypertriglyceridemia. It is more extreme in the cp/cp female, with triglyceride concentrations exceeding 1000 mg/dl at 9 months of age compared to 200 mg/dl in cp/cp males. There are also moderate elevations of low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions leading to increased concentrations of cholesterol and cholesteryl esters. The presence of both the hyperlipidemia and the hyperinsulinemia appears to be necessary for the development of the vascular and myocardial lesions in the JCR:LA-cp strain, neither being sufficient in itself. Thus the origin of the excessive VLDL concentrations in the corpulent rat is an important question. The two principal possibilities are a defect in lipoprotein lipase activity or function leading to impaired clearance of VLDL or an increased rate of hepatic secretion overloading the peripheral clearance mechanism.

Intravascular injection of heparin releases lipoprotein lipase from endothelial cells causing a sharp increase in plasma lipase activity and resulting in rapid clearance of chyomicrons and VLDL from the plasma. Triton WR1339,
in contrast, inhibits lipoprotein lipase activity leading to an accumulation of VLDL at a rate reflecting net secretion from the liver.\textsuperscript{12} We report here on studies using these techniques to test the above two possible origins of the VLDL hyperlipidemia in the JCR:LA-cp rat.

**Methods**

**Animals**

The JCR:LA-cp rats were bred in our colony at the University of Alberta, a colony established with nucleus breeding stock generously donated by Carl T. Hansen, Veterinary Resources Branch, National Institutes of Health, Bethesda, MD. The colony is derived from the fifth backcross to the LA/N strain, whereas other colonies (including that at the National Institutes of Health) have been taken to 12 backcrosses. The colony has been maintained as an outbred closed colony since establishment. The rats were bred cp/cp and +/+ of both sexes as previously described.\textsuperscript{6,7,13} They were maintained in polycarbonate cages on wood chip bedding (Aspen chips, Northeastern Products, Warrensburg, NY) at 20°C and 40% to 50% relative humidity on a 12:12 hour light cycle. All rats were fed ad lib a standard rat chow, Wayne Lab Blox (C:18, fatty acyl chains; C:20, triglycerides with C-16:16:16 or C-14:16:18 fatty acyl chains; C:50, triglycerides with C-16:16:18 or C-14:16:20 fatty acyl chains; C:54, triglycerides with C-16:18:20 or C-16:16:22 or C-18:18:18 fatty acyl chains; C:56+, triglycerides with C-18:18:20 or C-16:18:22 fatty acyl chains or greater fatty acyl carbon number.

The results reported for cholesteryl esters, phospholipids, and triglycerides represent the sum of the concentrations of the particular molecular species. We also report separately on the individual triglyceride molecular species in appropriate instances.

Evans blue solution (5 mg/ml in normal saline) was used for plasma volume measurements.\textsuperscript{16} Injection of 20 \( \mu \)l of the solution per 100 g body weight was made directly into the exposed left jugular vein of rats prepared as for the Triton studies. Blood samples (2.5 ml) were withdrawn from the cannula in the right jugular vein before and 10 minutes after injection of the Evans blue, and heparinized plasma was separated. The blood removed was replaced with normal saline. The plasma samples were diluted 1:2 with normal saline, and the optical density was read with the control sample as blank at 625 nm in a Unicam SP.500 spectrophotometer. The plasma volume was calculated with a correction for the dilution of the plasma volume in replacement of whole blood by saline following the initial sample.

**Calculations**

The lipid concentrations as a function of time for individual rats were used to calculate the initial rates of net clearance or secretion. The data were fitted to a quadratic equation:

\[
\text{[lipid]} = a + b \cdot t + c \cdot t^2
\]

using a least squares method,\textsuperscript{17} where \( a, b, \) and \( c \) are constants, and \( t \) is time (in minutes). The resulting constant, \( b \), gives the initial slope of the time concentration curve and thus represents the initial rate of change of concentration for the lipid in question. Corrections were applied for the effect of the sample removed and volume replacement with saline. If the postheparin clearance is a first-order kinetic process, the following relationship should hold:

\[
b = d[\text{lipid}] / dt = k \cdot [\text{lipid}]
\]

Thus the rate constant, \( k \), which is independent of the concentration is given by Equation (3). The rate constant

\[
\text{[lipid]} = a + b \cdot t + c \cdot t^2
\]
Table 1. Body Weights and Plasma Volumes of 3-Month-old JCR:LA-Corpulent Rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Plasma volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>5</td>
<td>511±7.4</td>
<td>11.4±2.5</td>
</tr>
<tr>
<td>+/+</td>
<td>5</td>
<td>315±5.6</td>
<td>10.4±2.0</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>5</td>
<td>387±8.6</td>
<td>9.88±1.40</td>
</tr>
<tr>
<td>+/+</td>
<td>6</td>
<td>192±4.7</td>
<td>7.45±0.93*</td>
</tr>
</tbody>
</table>

The values are the means±SD.
*p<0.01, +/+ vs. cp/cp.

Table 2. Initial Whole Plasma Lipid Concentrations in JCR:LA-Corpulent Rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Cholesterol</th>
<th>Cholesteryl esters</th>
<th>Phospholipids</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>6</td>
<td>33.6±8.1</td>
<td>144±30.5</td>
<td>233±59</td>
<td>344±180</td>
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<tr>
<td>+/+</td>
<td>5</td>
<td>14.4±5.3</td>
<td>62.5±8.1</td>
<td>69.1±11.8</td>
<td>19.6±5.2</td>
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<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>7</td>
<td>23.6±4.3</td>
<td>101±14.8</td>
<td>226±17.1</td>
<td>523±159</td>
</tr>
<tr>
<td>+/+</td>
<td>6</td>
<td>19.0±2.3</td>
<td>73.7±4.6</td>
<td>95.1±12.1</td>
<td>32.5±12.2</td>
</tr>
</tbody>
</table>

*The lipid concentrations are for 3-month-old rats in mg/dl (means±SD).
Figure 1. Whole plasma lipid concentrations (means±SD) in cp/cp male rats (n=6) after administration of heparin. The lines for the calculation of the initial rate of clearance as described in the text are shown and the results were: A. Cholesterol, b=-0.63. B. Cholesteryl esters, b=-2.31. C. Phospholipids, b=-6.32. D. Triglycerides, b=-27.3. All units are mg/dl/min and are based on the mean concentrations shown in the figures. Thus, they are not identical to the results from individual animals shown in the tables.

Figure 2. Whole plasma lipid concentrations (means±SD) in cp/cp male rats (n=5) after administration of Triton WR1339. See Figure 1 legend for details. The initial secretion rates were: A. Cholesterol, b=0.07. B. Cholesteryl esters, b=-0.18. C. Phospholipids, b=2.09. D. Triglycerides, b=9.52. The units are mg/dl/min and are based on the mean concentrations shown in the figure.
The calculated rate constants for postheparin clearance of phospholipids and triglycerides, $k$ in Equation (3) derived from the raw data in Tables 2 and 3 for $cp/cp$ rats, are shown in Table 4. The rate constants for cholesterol and cholesteryl esters were smaller ($3.6$ to $12.6$ min$^{-1} \times 10^{-3}$) and not significantly greater than zero. Those for both phospholipids and triglycerides were significant for all groups. The rate constants showed no significant differences between male and female animals.

Table 5 shows the net secretion of phospholipids and triglycerides in milligrams per minute. The net rate of secretion of cholesterol and cholesteryl esters was low, and the small changes that occurred over the short time period of the experiment lead to nonsignificant or even negative results. However, the net secretion of triglycerides was much greater in the $cp/cp$ rats and significantly greater in $cp/cp$ females than $cp/cp$ males. The initial concentrations and net secretion rates of the triglyceride molecular species were different before and after heparin administration as shown by the results in Tables 6 and 8. The fractional (or percentage) composition of the triglycerides by molecular species was calculated before and after treatment with heparin and Triton. The ratio of the final to the initial fractional composition for each triglyceride molecular species gives an index of compositional change. The results in Table 8 show that the $cp/cp$ rats showed a marked variation between molecular species ($p<0.001$, both sexes) with a significant relative increase in $C:48$ triglyceride molecular species after heparin ($p<0.001$, both sexes). The effect was more marked in the male $cp/cp$ rats than in the females ($p<0.05$). There was a concomitant decrease in $C:50$ and greater molecular species but similar secretion rates for $C:48$ and $C:50$ males but similar secretion rates for $C:48$ and $C:50$ molecules.

Table 1. Initial Rates of Change of Concentration of Plasma Lipids

Table 4. Calculated Rate Constants for Postheparin Lipid Clearance from Whole Plasma of JCR:LA-Corpuent Rats

Table 5. Net Rate of Secretion of Plasma Lipids In JCR:LA-Corpuent Rats

The values are the means±SD of the Initial rate of clearance calculated for each rat. The negative values indicate decreases in concentration. $P$ values indicate increasing concentration. $*p<0.05$, $p<0.001$ vs. control; $\dagger P<0.001$, $cp/cp$ vs. $+/$, $§p<0.05$ males vs. females.

Table 3. Initial Rates of Change of Concentration of Plasma Lipids

Table 2. Calculated Rate Constants for Postheparin Lipid Clearance from Whole Plasma of JCR:LA-Corpuent Rats

The values are the means±SD (mg/dl/mln) of the rate of change of concentration calculated for each rat. The negative values indicate decreasing concentration.

$p<0.05$, $tp<0.01$.
These differences between molecular species were also highly significant (p<0.001).

**Discussion**

The results from the lipid secretion and clearance rates are normally presented as amounts per 100/g of body weight of the animal. However, the great differences in body weight of the lean and corpulent rats were not associated with major differences in lean body mass or plasma volume. Thus, we feel it would be misleading to express the results in terms of body mass, and we have reduced our concentration changes to rate constants or values less than 1.0, a decreasing fractional concentration.

The results for postheparin plasma lipids indicate a marked clearance of VLDL. Figure 1 shows that the clearance of triglycerides from the plasma was complete
in 15 minutes. This, together with the quantitative data in Tables 3 and 4, makes it clear that the lipoprotein lipase of the cp/cp rat is readily released from the endothelium by a low dose of heparin and that the released enzyme is effective in mediating triglyceride hydrolysis. The male and female rats are similar in this regard. The data in Table 7 suggest a preferential activity toward the triglycerides with longer-chain fatty acids.

Administration of Triton WR1339 caused a buildup of VLDL as reported by other investigators. Abrams and Cooper12 predicted their studies on a "demonstrated linear increase in serum triglycerides for 90 minutes after administration of Triton WR1339." The results in Figure 2 demonstrate that this is not the case in the cp/cp rat. However, our curve-fitting procedure allows calculation of the initial rate of change, and thus the steady-state assumption is not necessary. The initial rates of change in the cp/cp rats were much higher than in the lean rats, indicating a much higher net hepatic secretion of triglyceride. The data in Table 5 show that the significant net hepatic secretion is confined to triglycerides. The female cp/cp rats also had markedly higher net secretion rates, which is consistent with their greater plasma triglyceride levels. While the Triton technique is a relatively simple method that yields information on net concentration changes, the results are unequivocal, and it is evident that the hyperlipidemia is due to a hypersecretion of VLDL.

An analysis of the secretion rate by triglyceride molecular species in Table 6 shows that the higher rate of secretion in cp/cp females is confined to the longer-chain fatty acids. The cp/cp male rat preferentially clears the fatty acids. The cp/cp male rat preferentially clears the fatty acid at the sn-2 position. Ridgeway and Dolphin18 have recently reported that both normal and hypothryroid Long Evans rats show preferential hydrolysis of short-chain fatty acids by lipoprotein lipase. This leads to sequestration of long-chain fatty acids in the intermediate density lipoprotein and LDL fractions. Nilsson et al.19 have suggested that such effects may be due to inhibition of hydrolysis of the preferred sn-1 or sn-3 acyl groups by the presence of a long-chain fatty acid at the sn-2 position. The observations reported here refer only to net clearance from the plasma compartment and reflect the sum of both hydrolysis of VLDL and uptake of hydrolysis products and remnant particles. Since the compositions of the major lipoprotein density classes were not measured, the whole serum values may not reflect the triglyceride molecular species composition of particular classes.

The characteristics of lipoprotein lipase may also be altered after release from its normal physiological site of action on the capillary endothelium. These factors, together with the major metabolic differences between the JCR:LA-cp rat and other strains, make the present observations complementary to those of Ridgeway and Dolphin18 and Nilsson et al.19. The corpulent rats may be compared to the fatty (fa/fa) Zucker rat, the other strain of obese rats. The fatty female has markedly lower plasma triglyceride concentrations (87.4±3.6 mg/dl) than either male or female cp/cp rats.20 The total cholesterol concentrations in both fatty and lean Zucker rats are similar to those of cp/cp and +/+ rats of our strain. Petit et al.20 have reported VLDL secretion of fa/fa and Fa/fa (lean) female rats to be 0.013 and 0.0045 mg/min, respectively. These values, which were obtained from perfused livers, are less than 1% and 3% of the rates we report for cp/cp and +/+ female rats in vivo. While there are major differences in technique, the results are consistent with the different fasting plasma concentrations of triglycerides in the two strains.

Taken together, the results show that the VLDL hyperlipidemia of the cp/cp rat is due to hepatic hypersecretion of the VLDL. The higher concentrations in the cp/cp female appear to be due to a greater secretion rate and perhaps to an overwhelmed lipoprotein lipase capacity, leading to a lower calculated rate constant for clearance. Identification of hepatic hypersecretion of VLDL as the source of the hyperlipidemia leaves open the further question of the mechanism of induction of the hypersecretion. This might be related to the hyperinsulinemia and impaired glucose tolerance of the corpulent rat.9,10

Certainly, a number of experimental manipulations of the rats, including food restriction and intensive exercise, reduce both the plasma lipid and insulin concentrations.21 However, the plasma VLDL levels of corpulent females are markedly higher than those of males, while their insulin resistance is much less severe.9,10 There is evidence that both activity of and mRNA expression for glycerophosphate dehydrogenase are increased in the cp/cp rat (Shillabeer, Hornford, Russell, Wong, and Lau, unpublished results). Thus, modulation of hepatic lipogenic enzymes may play a fundamental role in pathogenesis of the corpulent rat. In addition, there are clear differences in the metabolism of triglyceride molecular species in cp/cp rats that may have important pathophysiological consequences. The solution to these problems will be important to understanding the regulation of lipid secretion and concentrations. It may have implications for treatment of humans with insulin resistance, hypertriglyceridemia, and susceptibility to atherosclerosis.

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References

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