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 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 mechanisms. 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. 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. 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 (Continental Grain, Chicago, IL) with tap water available at all times. All rats were studied at 3 months of age.

Experimental Procedures

Rats were fasted overnight and anesthetized with pentobarbital (40 mg/kg body weight). The dosage was increased for corpulent rats to achieve and maintain an adequate depth of anesthesia. The jugular vein was cannulated with Silastic tubing (0.51 mm i.d., Dow Corning, Midland, MI). The tip of the cannula was positioned 1.0 cm superior to the right atrium, and the cannula was filled with normal saline. Triton WR1339 (Tyloxopal, Sigma Chemical, St. Louis, MO) was dissolved in normal saline at 0.11 g/ml. After initial blood sampling, 0.2 ml of this solution per 100 g body weight was injected into the jugular cannula and flushed with normal saline to a total volume of 1.0 ml. Sodium heparin solutions (Hepalean, Products, Warrensburg, NY) at 1000 U/ml. This was administered in the same manner as the Triton solution at 0.1 ml/100 g body weight. Blood samples were taken before injection of the heparin or Triton solution; at 5, 10, 15, and 30 minutes for the heparin experiments; at 30, 60, 90, and 120 minutes for the Triton experiments; and at 15, 30, 60, 90, and 120 minutes for control experiments. The latter experiments were identical to the others except that only normal saline was injected. The blood samples (1.0 ml) were taken into a syringe containing 1 mg each of ethylenediaminetetraacetic acid (EDTA) and phenylboronic acid (Sigma Chemical) in 0.05 ml of saline to inhibit plasma lecithin cholesterol acyltransferase and hepatic lipase. Immediately after removal of each blood sample, 1.0 ml of saline was injected into the venous cannula. Blood was centrifuged, and the plasma was separated immediately and frozen at −70°C until assayed. The plasma lipids were analyzed by using the total lipid profile technique of Kukstis et al. This technique involves preliminary digestion of the sample plasma with phospholipase C (Sigma Chemical) followed by derivatization with N, O-bis (trimethylsilyl)-acetamide (Pierce Chemical, Rockford, IL). Gas chromatographic analysis yields the concentration of free cholesterol, individual cholesteryl esters, diacylglycerols, and ceramides of the phospholipids and triglycerides with individual results by fatty acid carbon numbers. Thus the triglyceride molecular species may be specified according to their number of acyl carbon atoms as follows:

- C48, triglycerides with C-16:16:16 or C-14:16:18 fatty acyl chains;
- C50, triglycerides with C-16:16:18 or C-14:16:20 fatty acyl chains;
- C52, triglycerides with C-16:18:18 or C-16:16:20 fatty acyl chains;
- C54, triglycerides with C-16:18:20 or C-16:16:22 or C-18:18:18 fatty acyl chains;
- C56++, 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. Injection of 20 μl 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:

\[ [\text{lipid}] = a + b \times t + c \times t^2 \] (1)

using a least squares method, where \(a\) is a constant (initial concentration), \(b\) 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:

\[ b = d[\text{lipid}] / dt = k \cdot [\text{lipid}] \] (2)

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

\[ k = b / d[\text{lipid}] / dt \]
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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±1.4</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.

data for postheparin clearance given in the Results section have been calculated for each lipid component for each rat as per the equation:

\[ k = \frac{d[lipid]/dt}{[lipid]} \] (3)

Hepatic secretion of lipids is a more complex process and is not reducible to a simple rate constant. The data have been reduced from concentration changes to net secretion as mass of each lipid per minute. This data was also calculated for each rat separately based on plasma volume. The relative concentration changes in triglyceride molecular species were calculated as the fractional concentration of each species for the last sample (30 or 120 minutes after injection) divided by the fractional concentration in the initial sample. These calculations were performed for each rat individually. All data are presented as means and standard deviations. Statistical analysis was by analysis of variance with comparison of group means by unpaired t test.

Results

Table 1 shows mean body weights and plasma volumes. In spite of the large difference in body weights, cp/cp and +/+ male rats had similar plasma volumes and thus significantly different volumes per 100 g of body weight. The female rats showed an even greater difference in body weight between +/+ and cp/cp rats. However, the plasma volumes were much closer, and only the +/+ females were significantly different (lower volume) from the other genotypes.

The initial whole plasma lipid concentrations of rats of the four genotypes are shown in Table 2. These values are not significantly different from those previously reported for serum concentrations.11 The corpulent rats are characterized by elevations (approximately 100%) of both unesterified and esterified cholesterol. The phospholipid concentrations are more markedly elevated. The triglycerides, in contrast, show dramatically higher concentrations in the corpulent rats, with this being significantly greater in the females than in the males. Administration of heparin caused only nonsignificant decreases in cholesterol and cholesteryl esters, moderate decreases in phospholipids, and rapid clearance of triglycerides.

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>
</tr>
<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>
</tr>
<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).

The initial rates of change in lipid concentrations after injection of Triton WR1339 or heparin calculated individually for each rat are shown in Table 3. These results are for the constant b of Equation (1). Thus, they have units of mg/100 ml/min and are negative in the heparin experiments and positive in the Triton experiments. The calculated values of b for the control groups are small and, with one exception, not significantly different from zero. The data in Table 3 show that the changes in the concentrations of cholesterol and cholesteryl esters were generally small and not significant. This was true after both Triton and heparin injections and for all groups of rats. Phospholipids showed moderate changes, especially in the corpulent rats, with increases after Triton treatment that were not statistically significant. Decreases in phospholipid concentrations after heparin administration were larger but not significant compared to the control animals. The triglyceride concentrations showed large and significant changes. The postheparin rates of clearance were large and not significantly different between the male and female corpulent rats. The rate of increase in plasma triglyceride concentration after Triton administration was very high in the corpulent rats, and the rate of increase was greater in the females (p<0.05). The +/+ rats of both sexes showed much lower rates of increase than the cp/cp rats (p>0.001).
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 x 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 in the males (p<0.05). There was a concomitant decrease in C:50 and greater molecular species 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 variation in rate of secretion between triglyceride molecular species was highly significant (p<0.005) for all four genotypes. The cp/cp female rats had markedly higher secretion rates of C:52, C:54, and C:56+> molecular species than did cp/cp males but similar secretion rates for C:48 and C:50 molecular species. The results for the rate constants for postheparin clearance of triglyceride molecular species (Table 7) show that C:56+> triglycerides appear to be preferentially cleared from the plasma, compared to C:48 triglycerides, after release of lipoprotein lipase in both males and females. However, the variance was large enough that this apparent trend was not significant (p>0.05).

The relative concentrations of individual 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.
Table 6. Initial Concentrations and Rates of Secretion of Triglyceride Molecular Species in cp/cp JCR:LA-Corpulent Rats

<table>
<thead>
<tr>
<th>Molecular species</th>
<th>Concentration (mg/dl)</th>
<th>Secretion rate (mg/min)</th>
<th>Concentration (mg/dl)</th>
<th>Secretion rate (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C:48</td>
<td>19.1±1.5</td>
<td>0.033±0.024</td>
<td>28.5±6.1</td>
<td>0.028±0.028</td>
</tr>
<tr>
<td>C:50</td>
<td>47.8±12.4</td>
<td>0.152±0.073</td>
<td>81.6±26.4</td>
<td>0.163±0.057</td>
</tr>
<tr>
<td>C:52</td>
<td>95.7±20.5</td>
<td>0.245±0.165</td>
<td>213±64</td>
<td>0.529±0.190*</td>
</tr>
<tr>
<td>C:54</td>
<td>41.7±7.8</td>
<td>0.156±0.061</td>
<td>98.0±34.6</td>
<td>0.251±0.056*</td>
</tr>
<tr>
<td>C:56+&gt;</td>
<td>48.8±13.9</td>
<td>0.150±0.088</td>
<td>101±35</td>
<td>0.297±0.080*</td>
</tr>
</tbody>
</table>

The values for the concentrations are given in mg/dl and those for the secretion rates in mg/min (both means±SD).

Table 7. Calculated Rate Constants for Postheparin Clearance of Triglyceride Molecular Species from Plasma of JCR:LA-Corpulent Rats

| Genotype | C:48 | C:50 | C:52 | C:54 | C:56+>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male cp/cp</td>
<td>36.7±17.8</td>
<td>62.7±24.0</td>
<td>63.9±14.6</td>
<td>58.7±14.2</td>
<td>62.0±14.5</td>
</tr>
<tr>
<td>Female cp/cp</td>
<td>77.8±18.8*</td>
<td>94.1±31.7</td>
<td>91.3±21.0†</td>
<td>97.7±19.9†</td>
<td>120±26†</td>
</tr>
</tbody>
</table>

The values are in units of min⁻¹×10⁻³ (means±SD) and were calculated as the rate of clearance of each molecular species for each rat divided by the mean initial concentration of the species.

*p<0.05, females vs. males.

Table 8. Relative Concentration Changes of Triglyceride Molecular Species after Heparin and Triton Administration in cp/cp Rats

<table>
<thead>
<tr>
<th>Molecular species</th>
<th>Postheparin concentration (mg/dl)</th>
<th>Relative concentration change</th>
<th>Postheparin concentration (mg/dl)</th>
<th>Relative concentration change</th>
</tr>
</thead>
<tbody>
<tr>
<td>C:48</td>
<td>8.60±0.96</td>
<td>4.00±0.18</td>
<td>0.730±0.139</td>
<td>5.22±1.00†</td>
</tr>
<tr>
<td>C:50</td>
<td>3.75±1.07</td>
<td>0.803±0.052</td>
<td>1.256±0.027</td>
<td>7.55±2.68*</td>
</tr>
<tr>
<td>C:52</td>
<td>5.28±2.00</td>
<td>0.593±0.084</td>
<td>1.140±0.066</td>
<td>18.81±2.86†</td>
</tr>
<tr>
<td>C:54</td>
<td>3.26±1.39</td>
<td>0.739±0.142</td>
<td>0.931±0.044</td>
<td>11.10±4.37†</td>
</tr>
<tr>
<td>C:56+&gt;</td>
<td>5.50±8.61</td>
<td>0.791±0.289</td>
<td>0.748±0.080</td>
<td>15.11±7.10</td>
</tr>
</tbody>
</table>

All values are means±SD. The fractional composition of the triglyceride molecular species was calculated for zero time (data as in Table 6) and for 30 minutes after heparin (as above column 1) and 120 minutes after Triton administration. The relative concentration change is the ratio of the final to the initial fractional concentration. It is a dimensionless index of proportional change in the contribution of the triglyceride molecular species to the total triglyceride concentration. Values greater than 1.0 indicate an increasing fractional concentration, and values less than 1.0, a decreasing fractional concentration.

*p<0.02, t<0.01, t<0.001, male vs. female.

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 mass secreted per unit of time, thereby avoiding the confounding effects of the fat body mass. On this basis, direct comparison can be made between the cp/cp and +/+ rats in these experiments, since they were tightly controlled for age.

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 of concentration 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 triglyceride of shorter-chain fatty acids. Ridgeway and Dolphin18 have recently reported that both normal and hypothyroid Long Evans rats show preferential hydrolysis of short-chain triglycerides with longer-chain fatty acids. 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 lipoprotein lipase. 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.

Acknowledgments

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References


Index Terms: lipid clearance • lipid secretion rate • very low density lipoprotein • hyperlipidemia • JCR:LA-corpulent rat
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