Insulin Resistance and Impaired Glucose Tolerance in the Atherosclerosis-Prone LA/N Corpulent Rat

James C. Russell, Sunil K. Ahuja, V. Manickavel, Raymond V. Rajotte, and Roger M. Amy

The LA/N-cp rat is, when homozygous for the cp gene, hyperphagous, hyperlipidemic, and corpulent. The corpulent males develop atherosclerotic disease and myocardial lesions while corpulent females and lean rats do not. The fasting plasma glucose concentrations of corpulent rats are in the normal range, but insulin concentrations are markedly elevated in corpulent females and markedly elevated in corpulent males. Glucose tolerance testing reveals a glucose intolerance in corpulent rats in the presence of very high insulin concentrations, and this deficiency is more severe in the male rats. Glucagon concentrations are higher in corpulent rats than lean rats at 3 months of age and decrease progressively with age. In contrast, glucagon concentrations increase with age in lean rats and are higher than those in corpulent rats at 9 months. The islets of Langerhans of corpulent rats exhibit marked hyperplasia that increases with age. The hyperplasia is less extreme in corpulent female rats. The abnormalities suggest that this strain of rats has an insulin resistance leading to impaired glucose tolerance and progressive pancreatic disturbance. This process may be related to an accompanying defect causing elevated concentrations of very low density lipoproteins and correlates with the development of atherosclerotic disease. (Arteriosclerosis 7:620–625, November/December 1987)

A mutant strain of rats was isolated from a colony of SHR rats by Koletsky. This strain was, when homozygous for the mutant cp gene, corpulent, hyperphagous, hyperlipidemic, and hypertensive. The corpulent rats developed a fulminating atherosclerosis and had an average lifespan of 11 months. Two congenic strains have been developed from Koletsky’s original strain, incorporating the cp gene, the LA/N-cp and SHR/N-cp. The rats exhibit all normal characteristics of the base strain if homozygous normal (+/+), or heterozygous (+/cp). If homozygous for the recessive corpulent gene (cp/cp), they exhibit the corpulent phenotype and are hyperphagous, hyperlipidemic, and obese, but essentially normotensive. The hyperlipidemia is caused by a high concentration of an apolipoprotein B-poor very low density lipoprotein (VLDL) giving moderately elevated cholesterol concentrations and markedly elevated triglycerides. The hyperlipidemia is very similar to a human Type IV hyperlipidemia. The normotensive LA/N-cp rat does not develop the fulminating atherosclerosis shown by Koletsky’s original strain, but the rats do develop significant vascular and myocardial lesions. Russell and Amy have shown the presence of early atherosclerotic lesions identical to those found in other cholesterol-fed animal models for atherosclerosis. Both the vascular and myocardial lesions are age-dependent and largely confined to cp/cp males. Female cp/cp rats have even higher plasma lipid concentrations, but do not develop myocardial lesions, at least to 9 months of age.

Ellwood et al. have reported that the LA/N-cp rat has elevated plasma insulin levels, if cp/cp, but that fasting glucose concentrations were not significantly different in lean and corpulent rats. Michaels et al. found the SHR/N-cp rat to be hyperinsulinemic and to have an exaggerated response to an oral glucose load. These authors also reported that the fasting insulin levels increased in their immature rats between 4 and 9 weeks of age. The LA/N-cp rat was also shown by Michaels et al. to have similar high insulin levels and impaired glucose tolerance at 8 weeks of age, when corpulent. The Zucker fatty rat (fa/ra) also develops hyperplasia of the islets of Langerhans and insulin resistance with increased plasma insulin levels. This strain of rats is also obese and hyperlipidemic, and it has been reported that, in contrast to the LA/N-cp rat, a significant portion of the plasma lipids appears to be in the chylomicron and HDL fractions. The Zucker rat has not been reported to exhibit atherosclerotic or myocardial disease and is regarded as a model for the study of obesity. Direct comparison of the two strains in our laboratory has shown that both the magnitude of the hyperlipidemia and the impairment of glucose tolerance are much less in the fatty Zucker than in the corpulent rat.

We report a detailed study of insulin and glucose in the unmanipulated LA/N-cp rat. These animals show elevated plasma insulin from an early age with progressive hyperplasia of the β cells in the islets of Langerhans. Fasting plasma glucose concentrations are not radically different between lean and corpulent rats, but the corpulent rats have a variable impairment of glucose tolerance.
Methods

Lean and corpulent LA/N-cp rats were bred in the breeding colony in our laboratory as previously described. All procedures involving the rats were in accordance with the guidelines of the Canadian Council on Animal Care and were subject to prior institutional review. The rats were maintained in polycarbonate cages on a 12:12 hour light cycle at 20°C and 40% to 50% relative humidity. A rodent chow (Wayne Lab Blox, Allied Mills Inc., Chicago, Illinois) was available ad lib together with tap water. The rats were anesthetized in their own cage with 4% halothane in room air, and a surgical level of anesthesia was achieved on an operating table with 2% to 3% halothane. The chest was opened and the rats bled into a syringe from a needle puncture of the left ventricle of the heart. Heparin was used as anticoagulant and plasma samples preserved with aprotonin (500 kIU/ml). The pancreas was dissected free and the tail of the pancreas placed in Bouin’s solution for initial fixation, and transferred to formalin after 2 hours. Intravenous glucose tolerance tests were performed under pentobarbital anesthesia (0.04 mg/g body weight), following an overnight fast. A small incision was made over the femoral artery, which was freed by blunt dissection and cannulated with PE 50 tubing fitted with a three-way stopcock. The glucose load (0.5 g/kg body weight) was injected into the penile vein and 0.6 ml of blood withdrawn from the arterial catheter at 0, 1, 5, 10, 15, 30, 60, and 90 minutes. After removal of the erythrocytes the heparinized plasma samples were kept on ice until assayed for glucose and insulin. The blood was replaced with equivalent volumes of normal saline. The exponential rate constant for the first-order clearance of glucose from the plasma was calculated from the concentrations at 5, 10, 15, and 30 minutes.

Plasma glucose was measured by a glucose oxidase procedure (Glucose Analyzer, Beckman Instruments, La Jolla, California) for the glucose tolerance tests. A hexokinase procedure was used for other samples with an L. Multistat III centrifugal analyzer (Instrumentation Laboratories, Lexington, Massachusetts) and SmithKline Beckman reagents. Immunoreactive insulin was determined by double antibody radioimmunoassay with the use of rat insulin standards (Wellcome Reagents). Immunoreactive glucagon was determined by radioimmunoassay by using a single antibody precipitation method with standards of crystalline human glucagon (Ell Lilly and Company, Indianapolis, Indiana).

The pancreatic tissue was processed by standard histological techniques and embedded in paraffin. Sections were cut and stained for insulin and glucagon according to the methods of Sternberger with modifications. Sections were incubated with guinea pig antiserum to porcine pancreatic insulin (Dako, Cedarlane Laboratories, Hornby, Ontario, Canada) and rabbit antiserum to bovine pancreatic glucagon (Calbiochem, La Jolla, California). Link reagents were protein-A (E-Y Laboratories, San Mateo, California) and goat antirabbit IgG (Zymed, Cedarlane Laboratories) and developing reagent was rabbit antiperoxidase and peroxidase (Dako, Cedarlane Laboratories). Demonstration of immunolocalized peroxidase was performed according to Graham and Karnovsky.

Morphometric analysis was performed by using an automatic image analyzer (Image +, Dapple Systems, Sunnyvale, California). Insulin-stained slides were used and the islet size and volume density and the insulin-staining cell volume density calculated for each pancreatic sample. The technique is described in detail elsewhere. In brief, a gray-scale image of the pancreatic tissue was obtained, and by use of appropriate discriminator settings a binary image of the insulin-stained cells obtained. The ratio of this area to the similar area of all pancreatic tissue in the field of view is the B-cell volume density. The islet outline was then defined and the circumscribed space filled. The ratio of this area to the parenchymal area is the volume density of the islets. Islet size was determined as an oblate ellipsoid based on the major and minor axes.

Statistical analysis was performed by using analysis of variance and comparison of group means by unpaired t test.

Results

Table 1 shows the fasting plasma glucose concentrations of the rats as a function of age. Lean male rats had lower fasting glucose concentrations than corpulent rats at 1 and 3 months of age with this difference being significant (p<0.01) at 3 months. The rise in fasting glucose concentration by 6 months of age brought the plasma glucose of the lean male rats up to that of the corpulent rats. The glucose concentrations of corpulent female rats were significantly greater than those of the corpulent male rats at 1, 6, and 9 months of age (p<0.005). This was not evident at 3 months of age when the glucose concentrations were virtually identical.

The results in Table 2 show that lean rats exhibit low fasting insulin concentrations that increase slowly but significantly with age (p<0.001). The corpulent male rats have elevated insulin levels at 1 month (p<0.05) that increase rapidly to be 10 times those of the lean males at 3 months of age. Thereafter there was a slow but significant decrease with age (p<0.005, 9 vs. 3 months). The corpulent female rats at 3 months of age had elevated insulin levels, but these were significantly less than those of the corpulent males (p<0.005). While there was no change in insulin in the corpulent females at 6 months of age, the insulin level was significantly lower by 9 months of age (p<0.02) and lower than that in corpulent male rats (p<0.005).

The glucagon results are shown in Table 3. In male rats, the concentrations were significantly higher in the corpulent than in the lean animals at 3 and 6 months of age (p<0.001). However, the concentrations fell by 9 months of age in the corpulent males and rose consistently in the lean males so that at 9 months of age the concentration in lean rats was greater (p<0.001). The glucagon concentrations in cp/cp females were similar to the cp/cp males at 3 months, but did not drop with age. Consequently, at 9 months of age the females had higher glucagon concentrations than the males (p<0.001).

The results of intravenous glucose tolerance tests on 3-month-old rats are shown in Figures 1, 2, and 3. The responses of lean rats were consistent with a peak glucose
concentration at 1 minute of 467 ± 21 mg/dl, decreasing exponentially to 135 ± 17 at 90 minutes (Figure 1). There was a rapid insulin response to the intravenous glucose injection with a peak concentration of 141 ± 31 mU/l at 1 minute and return to resting values by 90 minutes. The mean value for the rate constant for glucose clearance, k, was 2.06 ± 0.64 × 10⁻²/min. The corpulent male rats, in contrast, showed both impaired glucose tolerance and a very variable response. Figure 2 shows peak glucose values similar to those of the lean rats (502 ± 31 mg/dl) and for most animals a slower exponential drop with time. The mean value for k was 0.94 ± 0.69 × 10⁻²/min, and this was significantly lower than the value for lean rats (p < 0.02). One rat, shown in Figure 1, after an initial drop showed a gradually increasing glucose level that was over 500 mg/dl at 90 minutes. This pattern has been observed repeatedly in corpulent male rats, occurring in approximately 20% of the population. The other rats had moderately elevated glucose concentrations of approximately 200 mg/dl at 90 minutes. The plasma insulin concentrations in the corpulent male rats in response to the glucose challenge were highly variable. Some rats developed extraordinarily high insulin levels; others showed little or no response above their elevated fasting level, or even a decrease. The overall response for the group was very limited, and the mean insulin concentration at 90 minutes was 507 ± 238 mU/l.

The corpulent female rats showed a response to the glucose challenge similar to that of the lean males. The plasma glucose clearance was equivalent to that of the lean males with a mean k = 2.29 ± 0.29 × 10⁻². Thus, while one animal showed a weak response in the first 30 minutes, the overall response for the group was very limited, and the mean insulin concentration at 90 minutes was 507 ± 238 mU/l.

**Table 1. Fasting Plasma Glucose in LA-N/cp Rats**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>+/+ male</td>
<td>cp/cp male</td>
<td>cp/cp female</td>
</tr>
<tr>
<td>1</td>
<td>105 ± 21 (5)</td>
<td>138 ± 43 (6)</td>
<td>187 ± 16 (6)*</td>
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<tr>
<td>3</td>
<td>116 ± 10 (6)</td>
<td>144 ± 18 (6)†</td>
<td>144 ± 20 (6)</td>
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<td>6</td>
<td>136 ± 12 (6)</td>
<td>137 ± 18 (6)</td>
<td>189 ± 23 (8)*</td>
</tr>
<tr>
<td>9</td>
<td>134 ± 24 (6)</td>
<td>125 ± 30 (5)</td>
<td>154 ± 3.4 (6)*</td>
</tr>
</tbody>
</table>

Values are means ± SD in mg/dl. Number of rats in parentheses.

"p < 0.05 vs. cp/cp male; †p < 0.01 vs. lean male.

**Table 2. Plasma Insulin Concentrations**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+ male</td>
<td>cp/cp male</td>
<td>cp/cp female</td>
</tr>
<tr>
<td>1</td>
<td>5.2 ± 1.8 (5)</td>
<td>32 ± 16 (6)</td>
<td>50 ± 28 (5)</td>
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<td>3</td>
<td>23 ± 8 (5)</td>
<td>425 ± 85 (5)*</td>
<td>229 ± 64 (6)†</td>
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<tr>
<td>6</td>
<td>24 ± 1 (5)</td>
<td>350 ± 115 (6)*</td>
<td>227 ± 101 (6)</td>
</tr>
<tr>
<td>9</td>
<td>52 ± 14 (5)†</td>
<td>239 ± 80 (5)*</td>
<td>127 ± 17 (6)†</td>
</tr>
</tbody>
</table>

Values are means ± SD in mU/l. Number of rats in parentheses.

*Concentration higher in corpulent than in lean males (p < 0.05); †concentration higher in males than in females (p < 0.005); §concentration higher at 9 months than at 3 or 6 months (p < 0.001).

**Table 3. Plasma Glucagon Concentrations**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+ male</td>
<td>cp/cp male</td>
<td>cp/cp female</td>
</tr>
<tr>
<td>1</td>
<td>186 ± 15 (5)</td>
<td>289 ± 90 (6)</td>
<td>175 ± 94 (5)</td>
</tr>
<tr>
<td>3</td>
<td>103 ± 9 (5)*</td>
<td>289 ± 13 (6)</td>
<td>255 ± 67 (6)</td>
</tr>
<tr>
<td>6</td>
<td>189 ± 22 (5)*†</td>
<td>298 ± 24 (6)</td>
<td>222 ± 19 (6)</td>
</tr>
<tr>
<td>9</td>
<td>207 ± 29 (5)*†</td>
<td>143 ± 27 (5)§</td>
<td>236 ± 24 (6)‖</td>
</tr>
</tbody>
</table>

Values are means ± SD in ng/dl. Number of rats in parentheses.

*Concentration lower in lean than in corpulent rats (p < 0.001); †concentration higher at 6 and 9 months than at 3 months (p < 0.001); §concentration higher in thin rats at 9 months (p < 0.001); ‖concentration lower at 9 months than at 3 or 6 months (p < 0.001); ‖‖concentration greater in females at 9 months (p < 0.001).
Figure 3. Plasma insulin and glucose response to intravenous glucose injection in cp/cp female rats aged 3 months. All conditions as for Figure 1.

Figure 4. Section of pancreas from lean male rat 3 months of age stained for insulin-secreting cells shows small discrete islets of Langerhans.

Figure 5. Section of pancreas from corpulent male rat at 3 months of age stained for insulin-secreting cells shows enlarged islets.

Figure 6. Section of pancreas from corpulent female rat at 3 months of age stained with hematoxylin and eosin.

Figure 7. Section of pancreas from corpulent male rat 6 months of age stained for insulin-secreting cells shows three markedly enlarged islets with early fibrotic changes.

Figure 8. Section of pancreas from 9-month-old corpulent female rat shows somewhat enlarged islets of normal architecture and no fibrosis.
minutes, none showed the highly compromised response seen in some corpulent males, and the overall response of the group was very similar to that of the lean male rats. The difference in k values between male and female corpulent rats was significant (p<0.001). The plasma insulin concentrations in the corpulent females showed a fairly strong response from an initial moderately elevated level and returned to 98 ± 37 mU/l at 90 minutes.

Figure 4 shows a section from the pancreas of a 3-month-old lean male rat (+/+ ) stained for insulin-secreting cells. The islets of Langerhans are small and discrete. A similar section from a 3-month-old corpulent male is shown in Figure 5. The islets are significantly enlarged compared with those of lean rats. The hyperplasia is essentially confined to the insulin-secreting cells. Figure 6 shows islets of Langerhans in a female corpulent rat at 3 months of age. The islets are slightly larger than those of lean male rats, but the hyperplasia is less extreme than that in the corpulent male rats at the same age (Figure 5). Figure 7 shows a pancreatic section from a corpulent 6-month-old male rat. The islets are markedly enlarged compared with lean male rats, whose islets are not greatly different at 6 months from the pattern shown at 3 months. By 12 months of age the islets of the corpulent male rats have hypertrophied to such a degree that individual islets are not always distinguishable, and considerable fibrosis is evident. Figure 8 shows a hematoxylin and eosin-stained section of the pancreas from a 6-month-old female rat. The islets are smaller and have a normal structure without fibrosis, in contrast to those in Figure 7.

Morphometric analysis of the islets showed that at 3 months of age there were dramatic differences in the size of the islets of lean and corpulent male rats (Table 4). There was a slight increase in islet size between 3 and 9 months in the lean rats. The islets grew rapidly in the corpulent rats, to be 30 times the size in the lean rats at 9 months. Female corpulent rats showed islet sizes similar to those of corpulent males at 3 months. However, the islets did not show the rapid increase in size with age and were significantly smaller than those of the corpulent male rats at 6 months and at 9 months (p<0.01).

Table 5 shows the results of morphometric analysis for the volume density of insulin-stained cells in the pancreas. The volume density of insulin cells is very much greater in the corpulent male rats. However, at 9 months of age the increase in volume density of insulin cells is less extreme than the increase in islet size. The corpulent female rats exhibited a more modest increase in the insulin cell volume density with a significantly (p<0.01) lower volume density at 9 months.

Corpulent male rats showed a continuing increase in islet size and extent of fibrosis between 9 and 12 months of age (data not shown). This process continued to be markedly less severe in the corpulent female rats at 12 months of age.

Discussion

The lean rats, as expected, exhibit normal characteristics of insulin and glucose metabolism. The fasting concentrations of glucose, insulin, and glucagon are unremarkable. The intravenous glucose tolerance test showed a strong insulin response and a consistently effective clearance of the excess glucose. The pancreatic morphology is completely normal. This is consistent with the rats' normal lipid status and freedom from vascular and myocardial disease.6-9

In contrast, the corpulent male rats, while not clinically diabetic, have a seriously compromised glucose metabolism. The fasting glucose was significantly but not greatly different from the lean rats. In response to the intravenous glucose load, the concentration at 1 minute was similar to that in the lean rats, but the average further insulin response was modest, although some rats produced transient very high levels. Thus, glucose concentration fell slowly in some rats and actually rose with time in others, despite very high insulin levels, demonstrating a severe insulin resistance in these 3-month-old male rats. A k value of less than 1.0 x 10^-2 is considered to reflect a diabetic state. A mean of 0.94 x 10^-2 with a large variance implies that a significant number of corpulent rats had k values of less than 1.0 x 10^-2. Two of six corpulent male rats had k values below 1.0 x 10^-2, while no lean rats were below 1.5 x 10^-2. The female corpulent rats exhibited a more moderate hyperinsulinemia and a greater glucose tolerance than the males. While the plasma glucose concentrations did not return to fasted levels by 90 minutes, the insulin concentration returned to fasting values following a very strong response (k = 560 mU/l at 1 minute, Figure 3).

Chan et al.21 have reported the results of oral glucose tolerance tests on fatty and lean Zucker rats. While the oral glucose load is a less severe and physiologically different challenge, some comparisons with our results may be made. Fatty male Zucker rats showed virtually the same plasma glucose response curve as the lean rats. The significant difference was an exaggerated insulin response, peaking at about 350 mU/l and returning to 150 mU/l at 60 minutes. This response is very similar to the female corpulent rats, but quite different from the deficient response of

Table 4. Size of the Islets of Langerhans In LA/N-cp Rats

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Genotype</th>
<th>+/+ male</th>
<th>cp/cp male</th>
<th>cp/cp female</th>
</tr>
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<tr>
<td>3</td>
<td>0.9 ± 0.2</td>
<td>11 ± 2</td>
<td>8.8 ± 2.5</td>
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<tr>
<td>6</td>
<td>1.0 ± 0.1</td>
<td>31 ± 6</td>
<td>9.6 ± 2.9*</td>
<td></td>
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<tr>
<td>9</td>
<td>1.5 ± 0.3</td>
<td>47 ± 12</td>
<td>4.4 ± 2.2*</td>
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</tbody>
</table>

Values are means ± SEM in μm^3 x 10^6. Six rats in each group. *p < 0.01 vs. cp/cp male.

Table 5. Insulin Cell Volume Density In the Pancreas of the LA/N-cp Rat

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Genotype</th>
<th>+/+ male</th>
<th>cp/cp male</th>
<th>cp/cp female</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.60 ± 0.12</td>
<td>3.9 ± 0.28</td>
<td>4.1 ± 0.80</td>
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</tr>
<tr>
<td>6</td>
<td>1.30 ± 0.20</td>
<td>6.1 ± 1.5</td>
<td>5.3 ± 0.92</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.20 ± 0.16</td>
<td>9.6 ± 0.96</td>
<td>3.0 ± 0.74*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.01 vs. cp/cp male.
the male corpulent rats. Shino et al.22 also reported changes with age in the fatty Zucker rat. They showed marked hyperplasia of the islets of Langerhans, although not as extreme as we report for the male corpulent rat. Fasting insulin levels were more moderate, with a peak value of 250 mU/l at 24 weeks of age.

As the rats aged, the insulin level fell consistently in the corpulent males, remained fairly constant in corpulent females, and rose slightly in 9-month-old +/+ males. This rise was statistically significant (p < 0.001) but not large in comparison with the values in corpulent male rats. Glucagon concentrations fell by 9 months of age in the cp/cp males, rose in the +/+ males, and remained somewhat elevated in cp/cp females. The decreased glucagon levels in 9-month-old cp/cp males are accompanied by an approximately twofold hyperplasia of the glucagon-secreting A cells in the islets.19 The significance of these glucagon changes is not clear at this time, but must represent a response to the abnormal insulin status.

Michaelis et al.5 have previously reported that the SHR/N-cp rat is hyperinsulinemic and insulin-resistant even when lean. In contrast, Michaelis et al.1 suggested that the LA/N-cp rat was not insulin-resistant and hyperinsulinemic if lean, but was if corpulent. The studies by Michaelis et al.4 were performed on 4- and 8-week-old rats. Since rats mature sexually at 3 months, or 13 weeks,23 their rats must be considered to have been juvenile animals. This may explain some of the differences between our results. Michaelis et al.4 extended their glucose tolerance test over 3 hours and took only three samples. They did not report k values and failed to show the insulin response or exponential decay in any of their results. They also did not note the phenomenon observed in some of our corpulent male rats of rising glucose in response to glucose injection or a negative response.

While Michaelis et al.4 found fasting insulin and glucose levels similar to our results, Ellwood et al.10 found lower levels for both in lean rats. This may have been a result of the synthetic diet used in their study. It should be noted as well that the rats used in this laboratory are descended from the fifth backcross to the LA/N in development of the congenic strain. The studies reported by Ellwood et al.10 and Michaelis et al.4,5 used rats derived from the twelfth backcross. Thus, the two colonies, maintained in isolation, may differ genetically in a subtle way.

The morphologic changes in the islets of Langerhans are complex, and a detailed quantitative study is reported elsewhere.19 However, the differences between the lean and corpulent male rats are extreme, as shown in Figures 6 and 7. The size of the islets increases continuously up to 9 months of age, and this hyperplasia is largely confined to insulin-secreting cells. The islet fibrosis is a consistent finding and is very marked in old rats. There is no evidence of insulin or pancreatitis, and the pattern is suggestive of B-cell death and replacement with fibrous tissue. The plasma insulin concentrations, in contrast, do not increase with age, remaining very high and variable. Thus, the fasting insulin concentrations do not reflect the extraordinarily large number of insulin-secreting cells, which must secrete relatively less insulin as the rats age. In this regard the corpulent rat may resemble the fatty Zucker whose large islets are inefficient releasers of insulin.24 The less extreme hyperplasia of the islets in the female corpulent rats correlates with their lower insulin concentrations. Both of these findings are indicative of a less severe abnormality of insulin and glucose metabolism in the female rats.

Tulp et al.25 have subjected LA/N-corpulent male rats to a high-protein, high-fat "cafeteria diet." They reported that oral glucose tolerance was not affected, although only a 0.25 g/kg body weight glucose challenge was used. A large glucose dose, such as the 1.0 g/kg used by other investigators for oral glucose tolerance testing,21 might have revealed significant differences. The diet also induced an increase in serum total cholesterol, which Tulp et al.25 reported as being from an increase in the VLDL + LDL lipoprotein fraction cholesterol content. However, the fractionation into HDL and VLDL + LDL was performed by magnesium-phosphotungstate precipitation, a technique that does not yield clear-cut lipoprotein separation of rat sera.9 The increase in cholesterol was interpreted as a diet-induced atherogenic change. An alternative view would suggest that the changes were probably caused by a further increase in the VLDL hyperlipidemia9 in an already atherosclerosis-prone strain.

Normal (lean) rats fed a cafeteria diet become hyperphagous and develop an obese state with hyperinsulinemia and glucose intolerance.26 However, such rats develop only a mild degree of glucose intolerance and insulin resistance that is unlike that shown by the male corpulent rat. In common with the fatty Zucker rat and the obese (ob/ob) mouse,27 these animals resemble the female corpulent rat.14 None of these animals develop the atherosclerotic disease evident in the corpulent male rat, even though marked hyperlipidemia may be present.14 This suggests that the more severe glucose intolerance or insulin resistance may be required for atherogenesis. Preliminary results in our laboratory have shown that mild food restriction and protracted physical exertion prevents the hyperlipidemia, hyperinsulinemia, islet hyperplasia, and myocardial lesion development in corpulent male rats. Severe food restriction alone caused only limited changes. These unpublished results further suggest a relationship between the hyperlipidemia, insulin resistance, and atherosclerosis in the LA/N-cp rat.

Our results indicate that the abnormal glucose metabolism of the corpulent rat is established at 3 months of age and persists through the midlife of the animal (9 months). The corpulent female rats exhibit a much more mild disturbance of insulin and glucose metabolism. The period from 3 months onward in these rats is characterized by the progressive development of atherosclerotic and myocardial lesions8,9 that are confined to the corpulent males. While this process may be strongly influenced by the coexisting hyperlipidemia, we suggest, as have Stout et al.28 that hyperinsulinemia and poor glucose tolerance may also play a role. This would account for the relative protection of the corpulent females and be entirely consistent with the strong association between diabetes and atherosclerotic disease in man.

Acknowledgment

We appreciate the expert assistance of Dorothy Koeslag in managing the breeding colony of rats.
References

21. Chan CE, Pederson RA, Buchan AMJ, Tubesing KB, Brown JC. Gastric inhibitory polypeptide (GIP) and insulin release in the obese Zucker rat. Diabetes 1984;33:536–542

Index Terms: atherosclerosis • animal models • insulin resistance • glucose tolerance • LAn/cp rat
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