Defective Endothelium-Dependent Relaxation in the JCR:LA-corpulent Rat

C.J. McNamee, C.T. Kappagoda, R. Kunjara, J.C. Russell

Abstract Endothelium-dependent relaxation of the aorta was assessed in JCR:LA-corpulent rats, which are hyperphagous, hyperlipidemic, hyperinsulinemic, and obese and spontaneously develop atherosclerotic disease and myocardial lesions. The findings in corpulent rats (6 months of age) were compared with those in age- and sex-matched lean rats. Aortic rings were prepared and mounted in Krebs-Henseleit buffer in a conventional organ bath. The tissue was contracted with norepinephrine (10−6 mol/L), and relaxation was induced using acetylcholine, the calcium ionophore A23187, or bradykinin. The maximum relaxation to acetylcholine was impaired in corpulent male rats compared with lean rats, whereas relaxation in response to the calcium ionophore was similar in the corpulent and lean animals. Aortic rings from corpulent and lean female rats showed no differences in response to acetylcholine or to the calcium ionophore. Removal of endothelium resulted in the loss of relaxant response to acetylcholine and the calcium ionophore. The relaxant responses to sodium nitrite were not significantly different in the corpulent and lean male rats when deendothelialized tissues were examined, but the sensitivity to sodium nitrite was significantly lower in rings from corpulent male rats with intact endothelium. There were no differences in the response to bradykinin between corpulent and lean rats. These findings suggest that there is a specific impairment of endothelium-dependent relaxation in the corpulent male rat that is limited to that mediated by muscarinic receptors. The possibility that endothelium-derived contractile agents are secreted in the vessels of corpulent male rats cannot be excluded. (Circ Res. 1994;74:1126-1132.)

Key Words • JCR:LA-corpulent rats • vasospasm • endothelium-dependent relaxation • calcium ionophore A23187

Endothelium-dependent relaxation of arteries can be elicited by many agents1,2 that act to increase the myoplasmic concentration of cGMP and to induce the relaxation of contracted smooth muscle.3 One of the agents responsible for this phenomenon has been shown to be nitric oxide.4 Studies of the cholesterol-fed rabbit model of experimental atherosclerosis have shown that there is an impairment of endothelium-dependent relaxation,5,6 and a similar effect has been demonstrated in diet-induced hypertriglyceridemia.7 The aim of the present study was to determine whether endothelium-dependent relaxation was impaired in an experimental model of atherosclerosis in which atherosclerosis and ischemic myocardial lesions occur spontaneously.

These studies were undertaken in the JCR:LA-corpulent (JCR:LA-cp) rat, one of five strains incorporating the corpulent (cp) gene first isolated by Koletsky.8,9 This strain (originally referred to as LA/N-cp) is now designated as JCR:LA-cp. It differs to some degree from the fully congenic LA/N-cp rats, and the characteristics have been described previously.10,11 The derivation of these various strains of rats has been described in detail by Greenhouse et al.10 The rats of all strains, if homozygous for the cp gene (cp/cp), are hyperphagous, hyperlipidemic, insulin resistant, and obese. If heterozygous (cp+/) or homozygous normal (+/+), the rats are phenotypically lean and are not distinguishable from the parent strains. The strains derived from the spontaneously hypertensive rat (SHR/N) are hypertensive, as is the SHR/N, whereas those derived from the LA/N and Wistar-Kyoto (WKY/N) strains are normotensive. The severity of the metabolic abnormalities and the expression of symptomatic disease vary among the strains.12-15

Unmanipulated male cp/cp rats of the JCR:LA-cp strain develop atherosclerotic disease along with myocardial lesions that include cell loss and old organized scars.16 These lesions appear to be of ischemic origin. Lean male (cp/+ or c+/+) and female (corpulent or lean) rats are essentially unaffected. Both types of lesions increase in frequency with age. The corpulent rats have a marked very-low-density lipoprotein (VLDL) hyperlipidemia resulting in greatly increased triglycerides and moderately raised plasma cholesterol concentrations.17 This is due to hepatic hypersecretion, with consequent increases in low-density lipoprotein (LDL) and high-density lipoprotein (HDL).18,19 The rats exhibit hyperinsulinemia and impaired glucose tolerance, which (like the myocardial lesions) are more mild in the female corpulent rats than in the male rats.20 All genotypes of this strain are normotensive,14 providing an experimental model of cardiovascular disease without the confounding factor of hypertension. In contrast, SHR/N-cp, LA/N-cp, and WKY/N-cp rats do not exhibit either vascular or myocardial lesions.15 The SHHF/Mcc-cp strain (derived from the SHR/N-cp strain) is highly prone to cardiomyopathy and subsequent congestive heart failure.

The present investigation was undertaken to determine whether endothelium-dependent relaxation is im-

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paired in cp/cp rats compared with +/+ rats. Endothelial-dependent relaxation was elicited in rings of aorta by use of acetylcholine and the calcium ionophore A23187. The non–endothelium-dependent relaxation properties of aortic rings were examined with sodium nitrite and bradykinin.

**Materials and Methods**

**Animals**

The present study was undertaken with cp/cp and +/+ rats that were bred in our established JCR:LA-cp colony as previously described. The animals were housed in pairs in 48-cm × 36-cm × 16-cm plexiglass cages with stainless-steel wire tops. Temperature was maintained at 20°C, with a relative humidity of 40% to 50%. Food was Wayne Lab Blox (Continental Grain Co) and was available ad libitum. The rats were studied at 6 months of age, when their body weights were 674±13 and 370±5 g for cp/cp and +/+ males, respectively, and 536±79 and 226±5.3 g for cp/cp and +/+ females, respectively. Care and treatment of the rats was in conformity with the guidelines of the Canadian Council on Animal Care and subject to prior institutional approval as provided for in the guidelines.

**Measurement of Relaxation**

The rats were anesthetized with sodium pentobarbital (20 mg/kg IP). A midline thoracotomy was performed, and the thoracic aorta was removed. Excess connective tissue was excised from the aorta, and it was cut into rings ~5 mm long, with special care being taken to avoid contact with the lumen in order to preserve the endothelium. The endothelium was deliberately removed in some rings by inserting the tip of a small pin into the lumen of the ring and gently rolling the ring between the pin and a filter paper wetted with Krebs’ bicarbonate buffer for 20 seconds. The rings were suspended in tissue baths of 12-ml capacity containing Krebs’ bicarbonate buffer solution at a pH of 7.4. The solution was maintained at 37°C with the aid of a heater/circulator (model E15, Haake Mess Technik) and continuously aerated with a gas mixture containing 95% O₂/5% CO₂. The rings were mounted on two stainless-steel triangular clips, the lower clip being attached to a movable support and the upper clip to a force displacement transducer (model 797159-3, Kulite Semiconductor Products). The output of the transducer was recorded (model 8188-44 recorder, Gould Instruments). Before experimentation, the rings were stretched to an optimum basal tension of 1.5 g. This optimum basal tension was established on the basis of long–active tension curves determined in preliminary experiments using a fixed concentration of norepinephrine (10⁻⁷ mol/L). The preparations were left in the tissue bath for a period of 2 hours for equilibration, and the bath fluid was changed every 30 minutes.

**Experimental Protocol**

Experimental groups consisted of six male and six female rats, both +/+ and cp/cp. Four aortic rings were prepared from each animal. The endothelium was removed from one ring. The preparations were studied after a 2-hour period of equilibration. A preliminary series of rings were exposed to cumulative additions of norepinephrine to give concentrations in the range of 10⁻⁷ to 10⁻⁳ mol/L, and the contractile response was measured. For the study of relaxation, the rings were precontracted with 10⁻⁶ mol/L norepinephrine. After the contraction had reached a stable plateau, the endothelium–dependent relaxation in response to acetylcholine was examined by obtaining cumulative concentration–effect curves (range, 10⁻⁷ to 10⁻⁴ mol/L) in two intact rings and the deendothelialized ring. The calcium ionophore (range, 10⁻⁸ to 10⁻⁵ mol/L) was applied to the fourth (intact) ring. After completion of the curves, the bath fluid was changed, and the preparations were left for 30 minutes. The rings were recontracted with norepinephrine, and the relaxation in response to sodium nitrite (range, 10⁻⁷ to 10⁻⁵ mol/L) was examined in two intact rings and the deendothelialized ring. The remaining intact ring was either deendothelialized and the response to the calcium ionophore (range, 10⁻⁸ to 10⁻⁵ mol/L) was examined, or the response to bradykinin (range, 10⁻⁷ to 10⁻⁵ mol/L) was determined. The data reported refer to one ring from each rat under each condition.

At the end of each experiment, some rings were immersed in 2.5% glutaraldehyde in Mallonig’s buffer while in situ and under a basal 1.5-g tension. The fixed aortic rings were then cut into two pieces and prepared for scanning electron microscopy using techniques described previously. The other rings were stained with Sudan red for lipids and were examined by direct light microscopy to determine if visible vascular lesions were present.

**Drugs**

The pharmacologic agents used were as follows: acetylcholine chloride, calcium disodium EDTA (CaNa₂EDTA), norepinephrine bitartrate, the calcium ionophore A23187, sodium nitrite, and bradykinin (Sigma Chemical Co). The Krebs’ bicarbonate buffer solution was used. The composition (mmol/L) was: NaCl 116.0, KC1 5.4, CaCl₂ 1.2, NaHCO₃ 22.0, NaH₂PO₄ 1.2, glucose 10.1, MgCl₂ 1.2, and CaNa₂EDTA 0.023. Stock solutions of the drugs were prepared in distilled water and added directly to the organ bath. All concentrations are expressed as the final concentration in the tissue bath fluid.

**Statistical Analysis**

Results are shown as mean±SEM. Relaxation is expressed as a percentage of the force of contraction induced by 10⁻⁶ mol/L norepinephrine. The linear portions of the dose–response curves for the agent applied (following log transformation of the concentration) were compared by ANCOVA. The dose–response curves were also fitted by use of the program ALLFIT, the best-fit values of maximal response and ED₅₀ were determined, and the significance of differences between groups was established.

**Results**

Table 1 shows the important metabolic and physiological parameters of the four genotypes of 6-month-old rats. The cp/cp rats, both of sexes, ate far more and were markedly heavier than the +/+ rats. Systolic blood pressure did not differ between cp/cp and +/+ male rats and was normal on tail-cuff measurement. Overnight-fasted rats showed no differences in plasma glucose measurements, but insulin concentrations were grossly elevated, with the cp/cp males having a significantly greater hyperinsulinemia than the cp/cp females (P<.05). Whereas there were only limited differences in plasma total cholesterol, the cp/cp rats exhibited a marked hypertriglyceridemia that was more severe in the cp/cp female rats.

**Male Rats**

**Endothelium-Dependent Relaxation**

The ED₅₀ for contraction in response to norepinephrine was 7.6±1.6×10⁻⁵ and 4.7±1.4×10⁻⁸ mol/L for cp/cp and +/+ rats, respectively. The exposure of aortic rings from cp/cp and +/+ rats to 10⁻⁶ mol/L norepinephrine resulted in identical contractile responses of 1.1±0.1 and 1.1±0.1 g for cp/cp and +/+ rats, respectively. A concentration of 10⁻⁶ mol/L norepinephrine was used for all studies of relaxation. The dose–response
curves of the relaxation response to acetylcholine are shown in Fig 1. The response of rings from cp/cp rats was lower at all concentrations studied. The calculated maximal relaxation was significantly \((P<.005)\) lower than that of the rings from +/- rats, as was the observed relaxation at \(10^{-6}\) mol/L acetylcholine \((P<.05)\). The \(ED_{50}\) for cp/cp rats was highly significantly greater \((P<.005)\) than that for +/- rats. There was no relaxant response to acetylcholine in rings with the endothelium removed.

The relaxation of norepinephrine-induced contraction elicited by the calcium ionophore A23187 is shown in Fig 2. The maximal relaxation elicited did not differ between rings from +/- and cp/cp rats, and there was no significant difference in \(ED_{50}\). There was no relaxation of the rings after removal of the endothelium.

**Response to Sodium Nitrite**

Fig 3 shows the relaxation of aortic rings from +/- rats in response to sodium nitrite. Relaxation was essentially complete \((100\%\) in intact rings) at \(10^{-5}\) mol/L sodium nitrite, and the response was not significantly different in deendothelialized rings \((P>.05)\). Corresponding results from aortic rings from cp/cp rats are shown in Fig 4. The relaxation of the intact rings was not significantly different from that of rings from +/- rats. However, the relaxation response of the deendothelialized rings was greater than that of the intact rings, and the \(ED_{50}\) was significantly reduced \((P<.05)\).

**Response to Bradykinin**

The maximal relaxation in response to bradykinin did not differ significantly between cp/cp and +/- rats \((40.9\pm9.2\%\) and \(60.0\pm9.3\%,\) respectively). Similarly, the \(ED_{50}\) was not significantly different \((P>.05)\) for cp/cp and +/- rats \((1.51\pm2.94\) and \(7.46\pm7.62\times10^{-7}\) mol/L, respectively).

**Scanning Electron Microscopy**

Fig 5 shows representative scanning electron micrographs of the intimal surface of various aortic rings from both +/- and cp/cp rats. The fixation of the rings while still under tension resulted in uncontracted samples similar to those obtained by perfusion fixation of arteries at physiological pressures. Panel A shows the surface of aorta from a +/- male rat. The endothelial surface is intact, although there is indication of some developing...
cell damage. The surface of a deendothelialized ring from the same rat is shown in panel B. The exposed basement membrane is clearly visible, along with a few adherent fragments of endothelial cells. In panel C, the endothelial surface of an intact ring from a cp/cp rat is shown. The endothelium is very smooth and unbroken, and the marginal folds between individual cells can be seen (faint white outlines of cells). The numerous small stomata with, in some cases, blood cells present are a common but not uniform finding and do not appear to represent acute damage. Panel D shows the luminal surface of a deendothelialized ring from the same rat, showing the exposed basement membrane and some cellular debris at the top of the field of view. These micrographs are typical views, and deendothelialized rings were found to always be essentially free of endothelial cells (<5% remaining). Intact rings at the conclusion of the experimental period were typically >90%, and not <60%, covered by an intact endothelial layer. Significant atherosclerotic lesions were not found in the rings by either scanning electron microscopy or light microscopy of Sudan red-stained rings.

Female Rats

_Endothelium-Dependent Relaxation_

The rings from female rats, both +/+ and cp/cp, showed contractile responses similar to those from male rats in response to norepinephrine. As shown in Table 2, the maximal relaxant response to acetylcholine was somewhat less than that of the males; however, there was no significant difference between +/+ and cp/cp animals in maximal response or ED50. Similarly, there was no difference in response to the calcium ionophore A23187 (Table 2). Aortas from female rats of both genotypes showed a significantly greater ED50 for A23187 than aortas from corresponding males (P<.05).

_Response to Sodium Nitrite_

Female rats showed no difference in response to sodium nitrite between genotypes (Table 2). Deendothelialized rings from female rats were not studied.

Discussion

The cp/cp rat of the JCR:LA-cp strain not only provides a striking model of the obese/hyperinsulinemic/hyperlipidemic syndrome but also exhibits the vascular and myocardial disease that is strongly associated with the syndrome in humans. A major unresolved question is the relation between the metabolic abnormalities and atherosclerosis and the myocardial lesions. The present results show that the aorta of the cp/cp rat does have a defect in endothelium-dependent relaxation, as revealed by an impaired relaxation response to acetylcholine. No such impairment was seen in aortic rings from cp/cp female rats. Thus, the impaired endothelium-dependent relaxation is correlated with the incidence of both vascular and myocardial lesions, which are confined to the cp/cp male rat.15,16

These results in an animal model of non-insulin-dependent diabetes mellitus are complementary to studies of insulin-dependent diabetes in both animals and humans. The spontaneous diabetic BB Wistar rat has been shown to have impaired relaxation in response to both acetylcholine and A23187.22,23 In contrast, Tantamaria et al24 showed that the alloxa diabetic rabbit exhibits impaired relaxation in response to acetylcholine but a normal response to A23187. Similarly, exposure of normal rabbit aorta to extreme hyperglycemic conditions resulted in the inhibition of the relaxation response to acetylcholine but not of the response to A23187. Limited studies have been performed in human subjects with insulin-dependent diabetes, showing a normal vascular relaxation response to acetylcholine26 and reduced relaxation to methacholine.27 Our results are similar to those obtained in rabbits by Cohen’s group24,25 and by Johnstone et al27 in humans and are consistent with an impaired relaxation that originates in the endothelium. The somewhat differing results of Durante et al23 suggest that the insulin-dependent BB Wistar rat differs significantly from the non-insulin-dependent cp/cp rat.

The results in Fig 2, together with the unimpaired response to both norepinephrine and Bradykinin in the male cp/cp rat, confirm that the defect in relaxation
does not lie in the contractile/relaxation properties of the smooth muscle. Bossaller et al.\(^{28}\) have shown that atherosclerotic human coronary arteries exhibit an impaired endothelium-dependent relaxation response to acetylcholine but a normal response to A23187. Our parallel results establish a common link between these two examples of atherosclerotic vessels. This suggests that the defect in the cp/cp rat aorta is a specific muscarinic one, as Bossaller et al.\(^{28}\) concluded in the case of human vessels. Electron microscopic examination of the aortic rings confirmed the existence of viable endothelium in our experimental rings and the effective removal of the endothelium in the deendothelialized specimens. In the particular rings shown in Fig 5, the endothelial layer on the ring from the cp/cp rat appears to have more intact endothelium than that from the +/+ rat. Thus, the results are not simply due to direct damage or the absence of the endothelium but to a mechanistic or metabolic defect.

The response of the cp/cp rat to sodium nitrite was complete (100% relaxation) at higher concentrations, suggesting, together with the normal response to A23187, that the relaxation mechanism of the vascular smooth muscle is normal. This again parallels the results of the study of Bossaller et al.\(^{28}\) on atherosclerotic human artery. The greater sensitivity to sodium nitrite exhibited by deendothelialized rings from cp/cp rats may suggest the presence of endothelium-derived contractile agents in these animals. Such agents have been reported by other investigators\(^{29,30}\) as being released under certain circumstances. Our earlier results showing that nifedipine markedly inhibits the development of myocardial lesions in the cp/cp male rat is supportive of this suggestion. Nifedipine induces two major effects in the cp/cp rat: a 50% reduction in plasma triglyceride (and therefore in VLDL) levels and the inhibition of myocardial lesion formation.\(^{31}\) This would suggest that in this model calcium channel antagonists may be able to counteract the effect of naturally occurring contractile agents such as endothelin.\(^{32}\) The absence of any effect of long-term treatment with acetylsalicylic acid on the myocardial lesions of the cp/cp male rat would suggest that products of the cyclooxygenase system are not involved in pathogenesis in this animal model.\(^{31}\)

**Table 2. Relaxant Responses of Aortic Rings From Female Rats**

<table>
<thead>
<tr>
<th></th>
<th>Maximal Response, % Relaxation</th>
<th>ED(_{50}), mol/L×10(^{-7})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetylcholine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>26.1±3.7</td>
<td>0.85±0.46</td>
</tr>
<tr>
<td>+/+</td>
<td>31.8±3.8</td>
<td>2.62±1.16</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Calcium ionophore</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp</td>
<td>68.9±7.8</td>
<td>7.13±2.25</td>
</tr>
<tr>
<td>+/+</td>
<td>55.4±6.9</td>
<td>4.89±1.86</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Sodium nitrite</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cp/cp intact</td>
<td>100±0</td>
<td>29.4±4.9</td>
</tr>
<tr>
<td>+/+ intact</td>
<td>100±0</td>
<td>40.8±7.2</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

cp/cp indicates rats homozygous for the corpulent (cp) gene; +/+, homozygous normal rats; and intact, intact endothelial surface. Values are mean±SEM as calculated using the ALLFIT program.

P values have been calculated by the ALLFIT program using the whole data set and comparing mathematical fits. They represent the comparison of cp/cp to +/+ rats.

Fig 5. Representative scanning electron micrographs of the surface of aortic rings from male rats after incubation in tissue bath. A shows intact endothelium on ring from a homozygous normal (+/+); B, deendothelialized ring from +/+ rat; C, intact ring from rat homozygous for the corpulent (cp) gene (cp/cp rat); and D, deendothelialized ring from cp/cp rat. All micrographs were at the same magnification. Bar=50 μm.
Our parallel results with ethanol treatment showed the prevention of ischemic myocardial lesions, with an associated decrease of hyperinsulinemia but not of hyperlipidemia. The incidence of atherosclerotic lesions in the aortic arch was not significantly decreased in ethanol-treated rats (authors’ unpublished results), suggesting that the protective effect of ethanol may involve changes in vascular behavior, including contractility and relaxation. This is consistent with reports that chronic ethanol consumption leads to the enhanced release of endothelium-dependent relaxing factor by endothelial cells.

The impairment of endothelium-dependent relaxation must be secondary either to the metabolic abnormalities of the cp/cp rat or to early atherosclerotic damage consequent to the abnormal metabolism. In this, the cp/cp rat would appear to resemble the atherosclerotic human or rabbit. The absence of gross lesions on the aortic rings studied is not surprising, because the favored locations for large lesions in the rat are the lesser curve of the aortic arch, the ostia of the great vessels, and the aortic bifurcation. The development of both the vascular and myocardial lesions in the cp/cp rat does not correlate with the hyperlipidemia, although it is probably necessary for the development of advanced atherosclerosis. The sexual dimorphism of the metabolic abnormalities and of both vascular and myocardial lesions is an important component of this animal model that mirrors those seen in humans. The induction of ischemic myocardial lesions is sex-linked in that it is confined to the cp/cp male rats but is not dependent on gonadal steroid hormones. This is consistent with the failure of the cp/cp female rat to develop vascular or myocardial lesions despite a much greater hypertriglyceridemia. The cardiovascular disease is, however, highly correlated with the hyperinsulinemia. The defect in endothelium-dependent relaxation follows the same pattern, being confined to the cp/cp male rat. This suggests the presence of a common underlying factor, perhaps initial damage to the endothelium by elevated circulating insulin levels or episodic hyperglycemia as implied by the findings of Tasfamari. If correct, this hypothesis could explain important elements of the induction of cardiovascular disease in both insulin-dependent and insulin-resistant diabetes.

Acknowledgments

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References

29. Gebremedhin D, Koltai MZ, Pogatsa G, Magyar K, Hadhazy P. Differential contractile responsiveness of femoral arteries from...


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