Response of the Aorta of the Obese Zucker Rat to Injury

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The response of the thoracic aorta to balloon-catheter-induced endothelial denudation was studied in two animal models of diabetes: the obese Zucker rat and the streptozotocin-treated Wistar rat. The obese Zucker rat, an animal with a metabolic profile similar to that of noninsulin-dependent human diabetics, was characterized by excessive body weight, hyperinsulinemia, hyperlipidemia, and a mild increase in plasma glucose levels. In Zucker rats sacrificed 3 weeks after endothelial denudation, the cross-sectional areas of the fibrocellular intimal lesions were found to be approximately twice as large as those of the lean control. In all other respects, the morphology of the lesions in the obese rats, as assessed by both light and transmission electron microscopy, was similar to that of the lean control rats. In streptozotocin-treated Wistar rats, neither the cross-sectional areas nor the morphology of the intimal lesions differed from those in control rats. These results indicate that, in the obese Zucker rat, the response to aortic intimal injury is altered.


Individuals with diabetes mellitus are thought to be at greater risk of developing atherosclerotic vascular disease than nondiabetic members of the population. Various factors, such as hyperglycemia, hyperlipidemia, abnormal levels of circulating insulin, or altered platelet function may play a role in the development of macrovascular disease in diabetic individuals, but the exact combination of any of these factors and the possible mechanism by which they exert an effect remains unclear.

Studies of this problem are complicated by a relative lack of appropriate diabetic animal models that develop accelerated vascular disease. Most of the available models, such as the spiny mouse and Chinese hamster, have been found to have only minimal, if any, macrovascular disease. The black Cebilbes ape (Macaca nigra) is probably the most suitable model since it exhibits insulinopenia, very low density lipoprotein (VLDL) excess, and spontaneous atherosclerosis; however, the supply of these animals is extremely limited.

The lack of spontaneous macrovascular disease in many animal models of diabetes may be due in part to the absence of atherogenic factors other than hyperglycemia. These factors are often present in humans and may contribute to the development of macrovascular disease. However, the diabetes-like metabolic state of these animals, although not leading to spontaneous arterial lesions per se, could alter the response of the vessel wall to an additional factor such as intimal injury. Therefore, we applied aortic balloon-catheter injury, an established, standardized endothelial injury, to two contrasting models of diabetes: the obese Zucker rat and the streptozotocin-treated rat. The former model is characterized by excessive body weight, insulin resistance, hyperinsulinemia, and mild hyperglycemia. The streptozotocin-treated rat is severely hyperglycemic and hypoinsulinemic and tends to exhibit ketosis, loss of body weight, and hyperlipoproteinemia. We selected these models because the obese Zucker rat shows similarities with human maturity-onset (Type 2) diabetes, while the streptozotocin-diabetic rat has some characteristics of human juvenile-onset, or insulin-dependent (Type 1), diabetes.
**Methods**

**Animals**

Obese and lean female Zucker rats aged 4 to 5 months were purchased from Harriet Bird Laboratories, Stow, Massachusetts; the obese rats weighed from 400 to 530 g and the lean, from 200 to 270 g. Adult male Wistar rats were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. All animals were fed Purina Rat Chow and water ad libitum and allowed to acclimatize to their environment for 2 to 3 weeks.

Wistar rats were made diabetic by the intravenous administration of streptozotocin (40 mg/kg) dissolved in 0.01 M citrate buffer (pH 4.5). Control Wistar rats received injections of the buffer. All rats in these groups were operated on 2 to 3 weeks after the administration of streptozotocin or the buffer. An additional group of Wistar rats, which had also received injections of the citrate buffer, was restricted to a food intake of 4 to 8 g/day. This regimen was begun 1 week before surgery and continued until sacrifice for the purpose of controlling for the expected loss of body weight in the streptozotocin-treated group.

Wistar rats were also used to assess whether the administration of insulin could affect the size of the intimal lesion that results from balloon injury. Rats received intraperitoneal injections of protamine-zinc insulin (18 U/kg/day) beginning 1 week before balloon denudation and continuing until sacrifice. Control rats received injections of saline.

**Surgical Procedure**

The method used to remove the endothelium of the thoracic aorta was similar to our previously described procedure. For the current work, collateral experiments were performed in adult Wistar rats to determine the optimal ratio of the balloon circumference to the average vessel circumference that would ensure complete denudation with minimal medial damage and consistent intimal lesion formation. In a group of 21 rats (300 to 455 g body weight), we observed that balloons with a circumference between 9.7 and 12.9 mm produced complete denudation and lesion formation consistently in thoracic aortas between the third and eighth intercostal arteries (aortic circumference was 5.5 to 6.3 mm as measured after perfusion fixation and embedding). The effects, however, were inconsistent when smaller balloons were used.

With the animals under pentobarbital (30 mg/kg, i.p.) and ether anesthesia, the left carotid artery was incised, and a 9 cm-long balloon catheter inserted for a distance of 5 cm into the thoracic aorta. The balloon was inflated with water to a circumference of 11.3 to 11.9 mm and drawn back and forth 12 to 16 times over a distance of 3.0 to 3.5 cm. The balloon was then deflated and withdrawn, the carotid artery ligated, and the wound closed with surgical clips. The diameter of the inflated balloon was checked immediately before insertion and again after withdrawal. The aorta was perfused with Evans blue dye 1 day or 1 week after surgery in three randomly selected animals to evaluate the extent and completeness of endothelial denudation.

**Tissue Preparation**

A catheter was inserted via the left ventricle into the ascending aorta. Phosphate-buffered 4% formaldehyde/1% glutaraldehyde (pH 7.3) was perfused under a pressure of 90 to 100 mm Hg for 10 minutes followed by an additional 10 minutes of nonpressurized fixation. Aortic rings were taken at the levels of the third, fourth, fifth, sixth, seventh, and eighth intercostal arteries and were then processed for light and electron microscopy using standard procedures of epoxy embedding. Epoxy sections 1 μ thick were stained with 1% toluidine blue and 0.075% basic fuchsin for light micrographs, and sections 2 μ thick were stained with 1% azure II and 1% methylene blue for morphometric evaluation. Ultrathin sections were cut on a Reichert OMU2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Philips EM300 electron microscope. The sections used for transmission electron microscopy were selected from those tissue samples that showed, by light microscopic morphometry, a cross-sectional area similar to the mean cross-sectional area for the group to which the tissue sample belonged.

**Blood Chemistry Assay**

All animals were weighed at weekly intervals until sacrifice. Serum was obtained from nonfasted animals at sacrifice and frozen at −20° C until assay. Serum glucose was measured by the glucose oxidase method. Immunoreactive insulin was measured by a single antibody method. Total serum cholesterol was measured by the method of Wybenga et al. Triglycerides and free fatty acids were extracted from serum by the method of Folch et al. and quantitated by densitometry.

**Morphometry**

Images of the 2 μ-thick sections were projected onto the tracing easel of a MOP 3 (Zeiss) image analyzer and the magnification adjusted to × 90. Separate, cross-sectional areas of each
entire tunica media and each entire lesion were obtained by tracing the outline of the respective images. Those three sections that showed the largest areas of lesion were selected to obtain an accurate estimate of lesion size for each animal. Average values for the areas of media and lesion were calculated for each animal based upon these three sections.

Linear measurements of the thickness of each tunica media were taken with an eyepiece micrometer both underneath the thickest part of the intima and at an area adjacent to, but not under, the intimal lesion; at the same locations, the number of elastic laminae were counted.

Results

Data obtained from the Zucker rats are summarized in table 1. At sacrifice, the obese Zucker rats weighed from 440 to 560 g as compared with 205±275 g for the lean controls. The nonfasting levels of serum cholesterol, free (unesterified) fatty acids, triglycerides, glucose, and immunoreactive insulin were all significantly elevated above control levels.

The cross-sectional areas of the intimal lesions (table 1 and figure 1) that formed in the aortas of the obese Zucker rats were significantly greater than those of the corresponding lean Zucker rats by comparison of either the rings with the three largest lesions or all six cross sections.

The aortic circumference as measured by the internal elastic laminae averaged 5.71 ± 0.10 mm for obese Zucker rats and 5.42 ± 0.12 mm for lean Zucker rats, not a statistically significant difference. The cross-sectional area of the media was similar in the obese and lean Zucker rats. For both obese and lean rats, the thickness of the media directly underlying the intimal lesions was 16% ± 3% (± ± se) greater than that part of the media that was not overlaid by intimal lesions.

As seen by light microscopy, the fibrocellular lesions in both the obese and lean Zucker rats were qualitatively similar (figure 1), consisting predominantly of cellular material surrounded by an extracellular matrix. In cross section, the cells had a rounded and irregular appearance, but in longitudinal section they were elongated. Lipid droplets in the intimal lesions were rarely seen, were very small when present, and seemed to occur with equal frequency in both the obese and lean animals. The lesions were always located opposite the intercostal arteries. No lesions were seen near the orifice of the intercostals.

Electron microscopy of the fibrocellular lesions in the obese Zucker rats showed cells resembling smooth muscle cells, which contained 60 A° filaments and were surrounded by a basement membrane (figure 2). In contrast to the smooth muscle cells in the medias, those cells in the lesions showed increased amounts of cellular organelles, particularly rough endoplasmic reticulum. The extracellular material consisted of amorphous material, reticulated basement membrane, collagen fibers, and fragments of elastin. The ratios of cellular to extracellular material appeared similar in the obese and lean animals. Neither light microscopy nor electron microscopy revealed qualitative differences between the lesions of obese and lean Zucker rats.

Streptozotocin-treated Wistar Rats

Data obtained from the streptozotocin-treated Wistar rats are shown in table 1. These rats were hyperglycemic and glycosuric; no urinary ketones were found during the course of the study. The nonfasting serum levels of cholesterol and triglycerides were not significantly lower than those in the controls and were comparable to those present at the onset of the streptozotocin-induced diabetes. The serum-immunoreactive insulin levels were significantly reduced in the streptozotocin-treated animals.

The streptozotocin-treated rats showed the same intimal responses to balloon injury as did the untreated control rats. There was no difference in the size of the intimal lesions in the diabetic and control rats, nor in the morphology of the intimal lesions in the streptozotocin-treated, saline-treated, or food-restricted groups.

Insulin treatment of the 10 Wistar rats resulted in a reduction of serum glucose to 33 ± 5 mg/dl (± ± se); serum glucose in the 10 saline-treated rats was 170 ± 6 mg/dl. The insulin treatment did not affect the cross-sectional area of the intimal lesion (10.7 ± 1.1 mm² for insulin-treated groups and 11.8 ± 1.4 mm² for saline-treated groups).

Discussion

In most animal models of diabetes, extensive macrovascular disease does not seem to occur spontaneously, although these animals develop macrovascular disease if they are fed cholesterol,7,15,20-22 We tried to overcome this disadvantage by experimentally inducing internal injury and observing whether the response to injury is altered in the presence of diabetes. In nondiabetic animals, intimal injury caused by the use of a balloon catheter is followed by a well-characterized series of events including the interaction of platelets with the exposed endothelium, the migration and proliferation of smooth muscle cells into the intima, and the development of a fibrocellular plaque.9,23 Although there is some uncertainty about the extent of medial smooth muscle cell damage caused by the use of a bal-
Table 1. Aortic Morphometry and Metabolic Profile in Zucker and Wistar Rats

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Zucker rats</th>
<th>Wistar rats</th>
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<tbody>
<tr>
<td></td>
<td>Obese (n = 10)</td>
<td>Lean (n = 11)</td>
</tr>
<tr>
<td>Body weight at sacrifice (g)</td>
<td>484 ± 13</td>
<td>246 ± 7</td>
</tr>
<tr>
<td>Immunoreactive insulin (μU/ml)</td>
<td>187 ± 40</td>
<td>36 ± 11</td>
</tr>
<tr>
<td>Total plasma cholesterol (mg/dl)</td>
<td>127 ± 6</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>Free (unesterified) fatty acids (mg/dl)</td>
<td>11 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>386 ± 60</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>129 ± 7</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>Intima (cross-sectional area, mm²)</td>
<td>5.94 ± 1.26</td>
<td>2.71 ± 0.68*</td>
</tr>
<tr>
<td>Media (mm²)</td>
<td>32.03 ± 1.82</td>
<td>29.27 ± 0.51</td>
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*p < 0.04 (Student’s unpaired t test).
All values are \( \bar{x} \pm SE. \)

Figure 1. Sections of aortas from obese (A) and lean (B) Zucker rats sacrificed 3 weeks after injury showing the lesion near its greatest thickness, a portion of media, and the vessel lumen above the lesion. The bar represents 50 μm; the magnification is the same for both sections.
The obese Zucker rat and the streptozotocin-treated Wistar rat represent two contrasting models of diabetes. As earlier studies show, the obese Zucker rat is hyperinsulinemic and hyperlipidemic. The hyperlipidemia has been characterized by elevations in the levels of VLDL, LDL, and HDL. The data on the obese Zucker rats in our study are consistent with these results. The high levels of circulating insulin reported by others and by us in this study are in sharp contrast to the low levels in the streptozotocin-treated Wistar rats. When intimal injury was induced, the two models of diabetes showed different responses. The fibrocellular, intimal lesions that formed in the aortas of the obese Zucker rats were approximately twice as large as those found in the lean, control Zucker rats. Moreover, the administration of streptozotocin to Wistar rats failed to affect the size of intimal lesion.

At present we cannot relate the differences in lesion size in the obese and lean Zucker rats to any metabolic characteristics of the obese rats. Several possible correlations are suggested, however. It has been shown that lipoproteins stimulate the growth of smooth muscle cells in vitro. However, hyperlipoproteinemia in vivo did not increase the thickness of an intimal lesion caused by intimal injury in the carotid arteries. Hyperinsulinemia, which has been suggested as an independent risk factor in the development of coronary artery disease in humans and which has been reported to stimulate the proliferation of arterial smooth muscle cells in vitro, could possibly affect the lesion size in the obese Zucker rat. However, we could not change the size of the lesions in Wistar rats by treating them...
with high doses of a long-acting insulin preparation. Other factors, such as abnormalities in platelet function, may also play a role in macrovascular disease in diabetics, but no information is available regarding platelet function in Zucker rats.

The quantitative difference in lesion size between obese and lean Zucker rats was not associated with qualitative differences in the ultrastructure of either the cellular or extracellular components of the intimal thickening. Despite the presence of hyperlipidemia in the obese rats, we did not find increased numbers of lipid droplets or other morphologic evidence of an increase in the amount of lipid in either the media or the lesions in these rats. This observation excludes the gross accumulation of large amounts of lipid in the aortas 3 weeks after injury, although such accumulation might develop in lesions of longer duration.

Unlike the obese Zucker rat, the streptozotocin-treated Wistar rats did not show any alteration in the intimal lesions. We used a relatively low dose of streptozotocin, which produces marked hyperglycemia but no severe weight loss, ketosis, or hyperlipidemia. Studies show that higher doses produce hyperlipoproteinemia and affect platelet aggregation in vitro. We decided upon the nonketotic, hyperglycemic model since it is relatively stable metabolically and more suitable for studies of long duration.

We conclude that in the obese Zucker rat, an animal that exhibits hyperlipidemia, hyperinsulinism, obesity, and very mild hyperglycemia, the response of the aorta to balloon-catheter-induced endothelial denudation is altered in the direction of an increased lesion size without concomitant alterations in qualitative morphological features.

References


