Decreased Coronary Vascular Reserve in Watanabe Heritable Hyperlipidemic Rabbits

Jean-Paul Clozel, Hans Lengsfeld, Herbert Kuhn, and Hans R. Baumgartner

Watanabe heritable hyperlipidemic (WHHL) rabbits have severe hypercholesterolemia due to a genetic defect in their low density lipoprotein (LDL) receptors. Therefore, premature atherosclerosis of the large arteries develops. For example, lesions of the aorta are always present after 6 months of age. However, in the coronary arteries, lesions have not always been observed at this age.

Presence or absence of coronary lesions has previously been assessed by microscopic examination of sections of the ventricle. However, it is difficult to make serial sections of all the large coronary arteries especially at the origin because of the complex three-dimensional geometry of this region. Therefore, we decided to evaluate the hemodynamic consequences of the stenoses by measuring coronary vascular reserve. For this purpose, coronary blood flow was measured with radioactive microspheres before and after maximal pharmacological vasodilation. The minimal coronary vascular resistance measured after maximal vasodilation depends essentially on the total cross-sectional area of the coronary vascular bed and should provide an integrated measurement of the functional consequences of the coronary atherosclerotic lesions.

Moreover, by making corrosion casts and morphological studies, we showed that at 300 days, WHHL rabbits have severe coronary atherosclerotic lesions located mainly at the origin of the main coronary arteries. These lesions are most likely responsible for the dramatic decrease in the coronary vascular reserve that we observed in these rabbits.

Methods

Animals

All the experiments were performed on 100- and 300-day-old, normal, Burgundy or pure-bred WHHL rabbits. The WHHL rabbits were from a colony bred from two pairs of WHHL rabbits kindly provided by Dr. Watanabe. The animals were housed individually with free access to food (normal rabbit chow: Nafag 814, Nafag Ltd., Gossau, Switzerland) and water.

Measurement of Plasma Cholesterol

Plasma cholesterol was measured colorimetrically with an automated method.

Measurement of Coronary Blood Flow and Coronary Vascular Resistance

The rabbits were anesthetized with hexobarbital (3 mg/kg/min) for implantation of three catheters: one for injection of the radioactive microspheres into the left ventricle through the left carotid artery, a second for the reference blood sample withdrawal in the right femoral artery, and the third for injection of the drugs into the right femoral vein. The animals were allowed to recover for 3 hours before starting the experiment.

The coronary blood flow of the conscious rabbits was measured with the microsphere technique. About...
400,000 radioactive microspheres (New England Nuclear, Boston, MA), 15 ± 5 μm in diameter and labeled with either 95Nb or 48Sc, were suspended in normal saline plus 0.01% Tween 80, were sonicated for 10 minutes, and were agitated before injection to prevent aggregation. They were injected into the left ventricle. Fifteen seconds before injection of the microspheres labeled with the first isotope and for 75 seconds thereafter, a reference blood sample was continuously withdrawn from the femoral catheter at a rate of 2 ml/min, and the radioactivity in this 3 ml sample was measured. This procedure was repeated after injection of carbocromen using radioactive microspheres labeled with the second isotope to determine coronary vascular reserve (see below).

At the end of the study, animals were killed by pentobarbital injection and their hearts were removed. After 2 days of fixation in 10% formaldehyde, the hearts were carefully dissected and all the coronary arteries and pericardiac tissue were removed. The left ventricular, free wall was dissected from endocardium to epicardium in three layers of equal thickness. All samples were blotted, weighed, and transferred to a two-channel gamma counter (Kontron, model GAMMAmatic 2) for determination of radioactivity levels, which were corrected for spill-over between channels. Regional blood flow was calculated by using the following equation:

\[
\text{Regional blood flow} = \frac{MT \times Q}{MS}
\]

where \(MT\) = radioactivity of the microspheres per gram of tissue, \(MS\) = radioactivity of the microspheres in the blood reference sample, and \(Q\) = reference blood withdrawal rate.

Coronary blood flow was measured in the right and left ventricle and in the left atrium, but not in the right atrium, where the number of microspheres was not sufficient (less than 400) to precisely measure blood flow. Coronary vascular resistance was calculated as mean arterial pressure divided by coronary blood flow.

**Measurement of Coronary Vascular Reserve**

Coronary vascular reserve was evaluated in these rabbits by measuring coronary blood flow and coronary vascular resistance before and after maximal coronary vasodilation. Maximal coronary vasodilation was achieved by injecting intravenously 9 mg/kg of carbocromen (Intensain, Boehringer Mannheim, Mannheim, FRG). Preliminary studies had shown that in 300-day-old, normal Burgundy rabbits, as well as in WHHL rabbits, this dose of carbocromen is sufficient to abolish reactive hyperemia after complete occlusion of the left main coronary artery for 20 seconds (Figure 1). The abolition of the reactive hyperemia is the criterion usually considered to prove maximal coronary vasodilation. Minimal coronary vascular resistance was the variable used to estimate coronary vascular reserve.

**Corrosion Cast Studies**

The corrosion cast studies were performed in 300-day-old Burgundy and pure-bred WHHL rabbits. The rabbits were given heparin (100 U/kg; Liquemin, F. Hoffmann-La Roche & Company, Basel, Switzerland) intravenously to prevent clotting. They were sacrificed by a blow on the neck. The heart, including the first proximal centimeter of the thoracic aorta, was removed. Then the blood in the coronary circulation was flushed out by perfusing the heart for 15 minutes with Ringer solution at a pressure of 90 mm Hg using a Langendorff apparatus. Methacrylate plastic (Batson’s compound number 17, Polyscience, Warrington PA) was injected at a pressure of 50 to 120 mm Hg. In many rabbits, injection of the methacrylate caused a collapse of the aortic valves, which resulted in the filling of the left ventricular and atrial chambers as well. The casts were

![Figure 1](http://atvb.ahajournals.org/) Abolition of coronary reactive hyperemia after infusion of carbocromen (9 mg/kg, i.v.). Arterial blood pressure, heart rate, and coronary velocity (measured with a Doppler probe on the LAD) were recorded before, during, and after a 20-second occlusion of the LAD. After carbocromen, reactive hyperemia was no longer observed.
revealed by macerating the heart tissue in a concentrated solution of potassium hydroxide. Presence of a stenosis on the cast of the coronary arteries was defined by a decrease of more than 20% of the diameter of the vessel.

**Histology of Coronary Arteries**

Seven 100-day-old and nine 300-day-old WHHL rabbits and seven 300-day-old Burgundy rabbits were anesthetized with pentobarbital (30 mg/kg). A catheter was introduced into the abdominal aorta, and then the aorta and heart were perfused retrogradely with 40 ml of 0.1 M phosphate buffer (pH 7.4) followed by 2.5% glutaraldehyde in the same buffer at a pressure of 130 mm Hg. After the heart stopped beating, the perfusion was maintained for 15 minutes at a pressure of 80 mm Hg. The heart and the ascending aorta were dissected out, and small preparations of the main and descending coronary arteries were fixed by immersion in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4, 4°C, for 90 minutes). After rinsing overnight with 0.1 M cacodylate buffer containing 7% sucrose (pH 7.4, 4°C), fixation with 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4, 4°C, 60 minutes), dehydration with ethanol/propylene oxide, and embedding in Epon, semithin cross-sections (0.75 μm) were cut and stained with toluidine blue and basic fuchsin. Cross-sections from the left anterior descending coronary artery were examined at 100 μm intervals. In the left main coronary artery, only the presence or absence of lesions could be evaluated, but the lesions could not be measured quantitatively because of the variability of the orientation of this vessel at its origin.

**Statistical Analysis**

The two groups of 100- and 300-day-old WHHL rabbits were compared to the groups of normal Burgundy rabbits of the same respective age by Student's unpaired t tests. A p value of less than 0.05 was considered significant. All data are expressed as means ± SEM.

**Results**

**Animal Description**

Mean arterial pressure, heart rate, and the ratio of heart weight/body weight did not differ significantly between the four experimental groups (Table 1). Plasma cholesterol in the WHHL group was significantly higher than in the Burgundy group at 100 and 300 days. All the experiments were carried out following the guidelines of the American Physiological Society.

**Coronary Blood Flow and Coronary Vascular Reserve**

Before injection of carbocromen, the baseline values of coronary blood flow, endocardium/epicardium (endo/epi) ratio, and coronary vascular resistances were similar in the four groups (Table 2 and Table 3). Injection of carbocromen markedly increased the coronary blood flow in all four groups. However, at an age of 300 days, flow in the left ventricle increased by only 86% in WHHL rabbits, whereas it increased by 267% in the Burgundy rabbits (p < 0.01). This difference was comparable in every part of the myocardium (Table 2). The endo/epi ratio either before or after carbocromen was the same in both 300-day-old rabbit groups.

Minimal coronary vascular resistance measured after injection of carbocromen was higher in the 300-day-old WHHL rabbits than in either the 300-day-old Burgundy rabbits or in the 100-day-old WHHL rabbits (Table 3). This difference in minimal coronary vascular resistance was present in both ventricles.

The minimal coronary vascular resistance was highly variable from one WHHL rabbit to another (Figure 2). Some 300-day-old WHHL rabbits had a nearly normal minimal resistance, but four of the rabbits had minimal coronary resistances above 15 mm Hg/ml/min/g. These variations were not related to plasma cholesterol levels. The minimal coronary vascular resistance did not correlate with the plasma cholesterol levels (r = 0.49, NS).

**Corrosion Casts**

Because of technical variables such as injection pressure, plastic viscosity, and hardening time, no quantitative comparison could be made of the diameter of the vessels in the different casts. However, the distribution of the coronary artery branches and of the stenoses could be clearly seen (Figure 3). The distribution of the coronary arteries was similar in the Burgundy and the WHHL rabbits. The left main coronary artery was always dominant and its diameter at the origin was three to four times larger than the diameter of the right coronary artery. The circumflex coronary artery had a diameter two to three times larger than the diameter of the left anterior descending artery. The left anterior descending artery branched into two to four dia-
### Table 1. Selected Variables In Four Experimental Groups

<table>
<thead>
<tr>
<th>Rabbit strain</th>
<th>Age (days)</th>
<th>n</th>
<th>Mean arterial blood pressure (B)</th>
<th>Heart rate (beats/min)</th>
<th>Heart weight/ body weight (%)</th>
<th>Plasma cholesterol (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burgundy</td>
<td>100</td>
<td>8</td>
<td>94 ± 4</td>
<td>75 ± 4</td>
<td>256 ± 7</td>
<td>0.209 ± 0.004</td>
</tr>
<tr>
<td>WHHL</td>
<td>100</td>
<td>9</td>
<td>101 ± 6</td>
<td>82 ± 4</td>
<td>276 ± 13</td>
<td>0.218 ± 0.006</td>
</tr>
<tr>
<td>Burgundy</td>
<td>300</td>
<td>9</td>
<td>101 ± 2</td>
<td>90 ± 4</td>
<td>258 ± 11</td>
<td>0.203 ± 0.005</td>
</tr>
<tr>
<td>WHHL</td>
<td>300</td>
<td>8</td>
<td>92 ± 8</td>
<td>92 ± 7</td>
<td>250 ± 9</td>
<td>0.206 ± 0.005</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

B = before carbocromen; C = after carbocromen.

*p < 0.001 compared to normal Burgundy rabbits.

### Table 2. Coronary Blood Flow before (C) and after (M) Maximal Vasodilation with Carbocromen

<table>
<thead>
<tr>
<th>Rabbit strain</th>
<th>Age (days)</th>
<th>n</th>
<th>C</th>
<th>M</th>
<th>Coronary blood flow</th>
<th>Left ventricular free wall</th>
<th>Endo/epi ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left atrium</td>
<td>Endo, Meso, Epi</td>
<td></td>
</tr>
<tr>
<td>Burgundy</td>
<td>100</td>
<td>8</td>
<td>1.55 ± 0.15</td>
<td>2.99 ± 0.23</td>
<td>4.27 ± 0.46</td>
<td>4.90 ± 0.57, 4.61 ± 0.53</td>
<td>3.87 ± 0.40, 1.29 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>6.97 ± 0.61</td>
<td>14.34 ± 1.46</td>
<td>12.32 ± 1.45</td>
<td>11.93 ± 2.24, 14.87 ± 2.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>1.80 ± 0.20</td>
<td>3.30 ± 0.21</td>
<td>4.47 ± 0.30</td>
<td>5.22 ± 0.33, 5.68 ± 0.38</td>
</tr>
<tr>
<td>WHHL</td>
<td>100</td>
<td>9</td>
<td>4.56 ± 0.42</td>
<td>13.88 ± 1.11</td>
<td>11.51 ± 0.68</td>
<td>9.91 ± 0.97, 14.47 ± 0.98</td>
<td>16.39 ± 1.39, 0.67 ± 0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>1.10 ± 0.16</td>
<td>2.53 ± 0.37</td>
<td>3.36 ± 0.51</td>
<td>3.84 ± 0.53, 4.23 ± 0.57</td>
</tr>
<tr>
<td>Burgundy</td>
<td>300</td>
<td>9</td>
<td>6.97 ± 1.02</td>
<td>12.80 ± 1.46</td>
<td>12.35 ± 0.87</td>
<td>11.21 ± 1.51, 15.11 ± 1.75</td>
<td>15.36 ± 1.79, 0.72 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>1.39 ± 0.15</td>
<td>2.21 ± 0.18</td>
<td>3.44 ± 0.25</td>
<td>3.90 ± 0.28, 4.06 ± 0.25</td>
</tr>
<tr>
<td>WHHL</td>
<td>300</td>
<td>8</td>
<td>3.85 ± 0.81†</td>
<td>5.62 ± 0.99§</td>
<td>6.41 ± 0.97†§</td>
<td>6.16 ± 0.97†§</td>
<td>8.58 ± 1.47†‡</td>
</tr>
</tbody>
</table>

Values are ml/min/g expressed as means ± SEM. The endo/epi ratio was calculated by dividing coronary blood flow in the endocardium by that in the epicardium of the left ventricular free wall.

C = baseline. M = after maximal vasodilation with carbocromen.

* p < 0.01, †p < 0.001 compared to the normal Burgundy group at the same age.

### Table 3. Coronary Vascular Resistance before (C) and after (M) Maximal Vasodilation with Carbocromen

<table>
<thead>
<tr>
<th>Rabbit strain</th>
<th>Age (days)</th>
<th>n</th>
<th>Left atrium</th>
<th>Right ventricle</th>
<th>Left ventricle</th>
<th>Left ventricular free wall</th>
<th>Coronary vascular resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Endo, Meso, Epi</td>
</tr>
<tr>
<td>Burgundy</td>
<td>100</td>
<td>8</td>
<td>65.9 ± 8.4</td>
<td>32.4 ± 2.3</td>
<td>23.6 ± 2.5</td>
<td>20.6 ± 2.1, 21.8 ± 2.2</td>
<td>25.9 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>11.2 ± 0.9</td>
<td>5.8 ± 0.9</td>
<td>6.7 ± 0.8, 6.1 ± 0.8</td>
<td>5.9 ± 0.9, 5.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>70.6 ± 17.7</td>
<td>30.7 ± 1.4</td>
<td>22.8 ± 1.2, 19.4 ± 0.8</td>
<td>17.9 ± 1.0, 23.8 ± 1.4</td>
</tr>
<tr>
<td>WHHL</td>
<td>100</td>
<td>9</td>
<td>19.0 ± 1.8</td>
<td>6.2 ± 0.5</td>
<td>7.2 ± 0.2</td>
<td>8.7 ± 0.5, 5.6 ± 0.2</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>106.1 ± 12.7</td>
<td>45.0 ± 4.6</td>
<td>34.3 ± 3.9, 30.1 ± 3.3</td>
<td>26.6 ± 2.6, 35.9 ± 3.8</td>
</tr>
<tr>
<td>Burgundy</td>
<td>300</td>
<td>9</td>
<td>16.2 ± 3.3</td>
<td>7.7 ± 0.8</td>
<td>7.5 ± 0.4</td>
<td>9.5 ± 1.5, 6.6 ± 0.8</td>
<td>6.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>73.9 ± 12.6</td>
<td>42.4 ± 3.7</td>
<td>26.8 ± 1.7, 23.8 ± 1.9</td>
<td>22.9 ± 2.0, 27.4 ± 2.3</td>
</tr>
<tr>
<td>WHHL</td>
<td>300</td>
<td>8</td>
<td>33.1 ± 7.4*†</td>
<td>21.8 ± 4.8‡</td>
<td>16.3 ± 2.2‡</td>
<td>16.6 ± 1.7‡, 12.7 ± 1.9‡</td>
<td>13.9 ± 2.4†‡</td>
</tr>
</tbody>
</table>

Values are mm Hg/mln/g/ml expressed as means ± SEM.

C = before carbocromen, M = after maximal vasodilation with carbocromen, endo = endocardium, meso = mesocardium, epi = epicardium.

* p < 0.001, †p < 0.01 compared to the Burgundy group at the same age. ‡p < 0.01, §p < 0.001 compared to the same strain at 100 days.
gonals: the first diagonal branch often shared a common origin with the left anterior descending coronary artery. The casts of 13 of the 300-day-old WHHL rabbits were examined. Stenoses were present in the ascending aorta in nine WHHL rabbits (Figure 4). The right coronary artery and the left main coronary artery were stenosed at their origin in 11 and 12 WHHL rabbits, respectively. The left circumflex coronary artery was stenosed in seven and the left anterior descending coronary artery in only one of the rabbits. We observed stenoses of a diagonal branch or a marginal branch in two rabbits. Only one of the 13 rabbits had neither coronary nor aortic stenotic lesions that were grossly visible.

These stenoses often reduced by more than 70% the diameter of the vessel as shown in Figure 5. However, it was difficult to quantify precisely the stenoses since the lesions were not always regular and could be present on a long portion of the coronary artery (especially on the left main coronary artery), which made the extrapolation of a normal reference diameter difficult.

In addition, the casts of six 100-day-old WHHL rabbits and of six 300-day-old Burgundy rabbits were examined. No significant stenosis either on the ascending aorta or the coronary arteries could be detected in these rabbits.

**Histology**

Six of seven 100-day-old WHHL rabbits showed small, eccentric neointima formations in the left main coronary artery, with a reduction of the arterial lumen always less than 10% (Figure 6A). The neointima was formed mainly by roundish, macrophage-derived foam cells and connective tissue (Figure 6B). Sometimes elongated smooth muscle cells, some of them containing lipid droplets, were also visible (Figure 6C). In contrast to the lesions present at 300 days, no crystalline material was observed.

All nine 300-day-old WHHL rabbits showed a dramatic neointima formation containing crystalline material in the left main coronary artery with a severe reduction of the luminal cross-section (Figures 7A, 7B, and 7C). Of these nine rabbits, only three showed lesions in the left anterior descending coronary artery, located approximately 5 mm distal from its origin. In one rabbit, more than 50% of the luminal area of this vessel was reduced. The other two rabbits had only moderate lesions that reduced the luminal area by less than 50% (Figure 8). Crystalline material was not detected in the left anterior descending coronary artery. Neovascularization was present in both types of arteries (Figures 7C, 7D, and 8A) but was more pronounced in the left main coronary artery. The internal elastic lamina was fragmented (which made discrimination between the media and the neointima impossible). The neointima was separated from the arterial lumen by a continuous endothelial layer in the left main and left anterior descending coronary arteries. The seven 300-day-old Burgundy rabbits were free of lesions.

**Discussion**

The present study shows that at 300 days of age, WHHL rabbits have a severe impairment of their coronary vascular reserve, which is due to coronary stenoses located mostly in the proximal portion of the coronary arteries.

To evaluate the coronary vascular reserve, we measured the minimal coronary vascular resistance after maximal pharmacological vasodilation. When the coronary

![Figure 3](image-url). Distribution of the coronary arteries in a corrosion cast of a 300-day-old Burgundy rabbit. RCA = right coronary artery, LAD = left anterior descending coronary artery, LMCA = left main coronary artery, CX = circumflex coronary artery.

![Figure 4](image-url). Occurrence of coronary lesions in the casts of 13 WHHL rabbits, 300 days old. Abbreviations are explained in Figure 3 legend.
arteries are maximally dilated (which can be controlled by the absence of reactive hyperemia), the coronary vascular resistance depends exclusively on the total cross-sectional surface area of the coronary arteries. Measurement of the minimal coronary vascular resistance gives a global evaluation of the functional state of the coronary vascular bed.

Moreover, measurement of coronary vascular reserve is a more sensitive indicator of the presence of coronary stenoses than is the measurement of basal (before phar-
Figure 6. Light micrographs of a semithin cross-section of a left main coronary artery of a 100-day-old WHHL rabbit. A. Whole cross-section. The internal elastic lamina (IEL) is partly covered by an eccentric neointima (NI). M = media. Bar = 200 μm. B. Higher magnification of the neointima shown in A. The neointima is formed by foam cells located between the endothelial layer (EC) and the internal elastic lamina (IEL). Bar = 50 μm. C. Higher magnification of the area inside A. In the neointima, foam cells (FC) are preferably located near the internal elastic lamina (IEL). Also nonfoamy cells (arrow) are visible in the area that is rich in connective tissue (*). The neointima is covered by endothelial cells (EC). Bar = 50 μm.
Figure 7. Light micrographs of a section of a left main coronary artery from a 300-day-old WHHL rabbit. A. Whole cross-section. The internal elastic lamina (IEL) is often disrupted. The neointima (NI) contains crystalline material (C). L = lumen, M = media. Bar = 50 μm. B. Higher magnification of area inside A. L = lumen, SMC = smooth muscle cell, C = crystalline material. Bar = 50 μm. (Continued on next page.)
Figure 7. C. Higher magnification of area inside A. Note the neovascularization (*) in the media (M) and neointima (NI) and the fat-filled foam cells (FC). IEL = internal elastic lamina, SMC = smooth muscle cell. Bar = 50 μm. D. Higher magnification of area inside A. Numerous small blood vessels (*) are observed in the media (M) and the neointima (NI). IEL = internal elastic lamina. Bar = 50 μm.
macological vasodilation) coronary blood flow. It has been shown that coronary stenoses have to obstruct more than 90% of the cross-sectional area to reduce blood flow at rest, but in contrast, coronary stenoses of 50% are sufficient to decrease maximal coronary flow. Another advantage of the measurement of coronary vascular reserve is that it takes into account all the characteristics of the stenoses that can have a functional consequence, for example, the length and the shape of the stenoses that are important determinants of the limitation of coronary blood flow. A further advantage of the measurement of coronary vascular reserve is that it is a global measurement, in contrast with histological techniques, which are, of necessity, restricted to a limited number of sections. The corrosion casting technique allows the examination of all the coronary arteries. However, as mentioned before, coronary stenoses are difficult to quantify with this technique.

In the present study, despite the severe impairment of coronary vascular reserve, the endo/epi ratio was not significantly decreased in the 300-day-old WHHL rabbits compared to the 300-day-old Burgundy rabbits. This result confirms a previous study which has shown that ischemia does not decrease the endo/epi ratio in rabbits. This finding was also reported in pigs. In contrast, in dogs, ischemia decreases the endo/epi ratio. However, dogs are known to have a very high coronary collateral circula-
Atherosclerosis characterized by clusters of foam cells, intimal material in the neointima by alteration of the media, proliferation of smooth muscle cells, and presence of crystalline material in the neointima by alteration of the media and by fragmentation of the lamina elastica interna. These histologic findings are similar to what has been reported before. However, the incidence of coronary stenoses that we detected by using the corrosion cast technique was higher than that reported by Watanabe from inspection of histological sections from the same strain and at the same age. As mentioned before, by using corrosion casting, the whole coronary arteries can be visualized, whereas histological examination is restricted to a limited number of sections.

Coronary vascular reserve describes the ability of coronary blood flow to increase when myocardial oxygen consumption is increased in conditions such as stress or exercise. A decrease of coronary vascular reserve has been described in patients with chronic coronary artery disease. Many techniques to measure coronary vascular reserve in patients are now available and thus, the severity of coronary stenoses can be determined. Therefore, evaluation of coronary vascular reserve may represent a convenient method to estimate the global functional status of the coronary vascular bed in WHHL rabbits. Further studies are required to assess if measurement of coronary vascular reserve could be used to evaluate any effects that lipid-lowering and antiatherosclerotic drugs might have on the coronary vascular bed.

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