Failure of Swimming Exercise to Improve Capillarization in Cardiac Hypertrophy of Renal Hypertensive Rats

Karel Rakusan, Pierre Wicker, Marvan Abdul-Samad, Bernadine Healy, and Zdenek Turek

Female Sprague-Dawley rats were made hypertensive by the two kidney/one clip Goldblatt procedure, while control animals were sham-operated. One week later, half of the animals were subjected to a moderate swimming exercise and the other half remained sedentary. Thus, four experimental groups, each consisting of 14 rats, were formed: control animals that were exercised or kept sedentary and corresponding renal hypertensive animals either exercised or sedentary. In hypertensive rats, a significantly increased left ventricular weight and reduced coronary reserve were found. Cardiac hypertrophy in hypertensive rats was characterized by a lower number of capillaries on a tissue cross-section, larger heterogeneity of the capillary net, and a less uniform orientation of capillaries in space. Total length of capillaries in the hypertrophic hearts increased significantly, but less than the increase in cardiac weight, resulting in reduced capillary length density. Chronic swimming for 2 hr/day for a period of 6 weeks, subsequent to a 4-week acclimation period, did not significantly influence any of the investigated indexes of capillaries from hypertrophic hearts. In the normotensive rats, chronic swimming resulted only in a moderate increase in total capillary length associated with a small increase in the left ventricular weight of similar degree. Thus, chronic exercise in normotensive rats induced a moderate increase in total capillary length per left ventricle, while it did not alleviate impaired capillarization of hypertrophic hearts from hypertensive rats. (Circulation Research 1987;61:641–647)

Cardiac hypertrophy caused by chronic pressure overload is usually characterized by a decreased coronary flow reserve and reduced capillary supply. The only exceptions seem to be situations in which the overload took place during fetal development or in the neonatal period. Limited chance to increase the blood flow in the case of various stress situations, together with less favorable geometrical conditions for the oxygen supply, may be contributing factors to the transition from simple cardiac hypertrophy to cardiac failure. On the other hand, various reports suggest the possibility that physical training could beneficially influence the coronary flow reserve and improve the capillarization of both normal and hypertrophic hearts. It is important to mention that the studies reporting favorable effects of exercise on these two indexes are not unanimous as some reviews suggest. This point will be analyzed in the "Discussion" of this article.

The objective of the present experiments was to test whether chronic swimming can improve the poor capillarization of hypertrophic hearts from rats with renal hypertension. The capillarization was studied not only by traditional indexes such as capillary numerical density but also by the more advanced morphometric methods that yield estimates of the heterogeneity of capillary spacing, capillary length density, and total length of coronary capillaries. The results were compared with coronary blood flow measurements and estimates of the coronary flow reserve were obtained from the same experimental animals. The experimental design was as follows: sham-operated rats were subjected to chronic swimming or kept sedentary, and the results were compared with those obtained from renal hypertension rats that were also either exercising or kept sedentary. We found that chronic swimming, using our experimental protocol, did not improve capillarization of the hypertrophic hearts or increase their coronary flow reserve.

Materials and Methods

Forty-two young female Sprague-Dawley rats were used in these experiments. They were housed 2 or 3 per cage and fed standard rat chow (Purina Chow). At the age of 40–45 days, 2 kidney/1 clip Goldblatt renal hypertension was induced in rats under ether anesthesia by placing a silver clip on the left renal artery. Rats subjected to a sham operation served as controls. A week later, half of the renal hypertensive and half of the sham-operated animals were submitted to a regular exercise program following the protocol proposed by Ostman-Smith. This program consists of two phases: an acclimation period of 4 weeks, during which the rats were made to swim for increasingly longer durations from 15 minutes the first day to 120 minutes per day, followed by a stable training period of 5–6 weeks, during which the rats swam 2 hours a day, 5 days a week.
The rats were exercised in groups of 6–8 in a Plexiglas tank. Water temperature was maintained at 36°C. Blood pressure was measured at weekly intervals with an indirect tail-cuff method. Only rats with a systolic blood pressure consistently greater than 150 mm Hg were included in the group of renal hypertensive animals (approximately 40% of rats with the Goldblatt operation).

After 9–10 weeks of either the swimming program or cage confinement, coronary blood flow and resistance were measured in conscious, unrestrained rats as described previously. Briefly, a PE10 catheter was positioned in the left atrium several days before the procedure, and a PE50 catheter was placed into the abdominal aorta 2–3 hours prior to the measurements. The aortic catheter was used for blood pressure recording (MP 15 transducer) and for the withdrawal of the reference sample after the injection of microspheres, while the radioactive microspheres were injected into the left atrium (15 μm in diameter, labelled with either strontium 85 or cerium 141, at least 300,000 microspheres injected after previous sonification).

After the first set of microspheres was injected, a 50% carbochrome was infused at a constant rate of 0.136 mg/min to a total dose of 12 mg/kg, which was followed by the injection of the second set of microspheres 6–8 minutes later.

At the end of the experiment, the rats were anesthetized with sodium pentobarbital, and their hearts were perfused in situ with heparinized saline and fixed with 1.5% glutaraldehyde buffered to pH 7.4 with phosphate buffer. The perfusion pressure was 80–90 mm Hg; the perfusion time was approximately 20 minutes. Subsequently, the hearts were removed and trimmed of fat and fibrous tissue. The proper location of the left atrial catheter was confirmed. The ventricles were separated from the atria and weighed. The left ventricle and septum were cut at one third of the distance from the base to the apex of the heart, and samples were taken for morphology. Both the heart and reference blood samples were placed in a gamma counter (Packard 5010) together with reference blood samples and used for estimating the coronary blood flow and resistance.

Histological methods were used as described previously. Briefly, after fixation and dehydration in alcohol, the samples were embedded in historesin. Sections 1 μm thick were stained by Avivone’s silver methenamine method for staining basement membranes. Afterwards, photomicrographs were taken of the endomyocardial and middle regions of the left ventricular free wall. From both cardiac regions, 5–7 photomicrographs were taken, each having an area of 28,500 μm².

Morphometric evaluation was performed at three levels. First, traditional indexes of cardiac capillarization were determined: average capillary density (number of capillaries/mm²) and myocyte-to-capillary ratio. In both counting of capillaries and myocytes, the rule of Gunderson to correct for edge effect has been applied.

The second approach was to evaluate the heterogeneity of the capillary spacing by using our method of capillary domains. The principle of the method is to assign a surrounding area (domain) to each capillary on the tissue cross-section using an image analyzer and a special soft ware program. From the domain area, the equivalent radius of a Krogh tissue cylinder (R) with the same area is calculated. The frequency distribution of these radii is approximately log-normal. Thus, the log SD of the radii can serve as a heterogeneity index. A larger log SD indicates a more pronounced heterogeneity and vice versa.

Finally, capillary length density and the total capillary length were estimated by applying the short-cut estimation procedure proposed by Mathieu et al. In this case, the capillary length density \((J_A)\) is estimated using the numerical coefficient \((c_K)\) and numerical density \((N_A)\). For a transverse section, the numerical coefficient becomes \(c(K,0)\), and its values lie between 1, when the capillaries are straight segments completely aligned according to the axis of anisotropy, and 2, in the case of random orientation with no preferential direction of the capillaries. \(J_A\) is then computed by multiplying \(N_A,0\) with \(c(K,0)\). The degree of anisotropy is also expressed by the dimensionless constant \(K\), which is the concentration parameter of the Fisher axial distribution. An isotropic arrangement, i.e., random directions, yields a \(K\) equal to 1; the higher the values of \(K\), the higher is the degree of capillary orientation around a preferred axis, i.e., the degree of anisotropy. Both \(c_K\) and \(K\) values can be estimated by measuring the capillary numerical density on transverse \(N_A,0\) sections and longitudinal \(N_A,90\) sections; the ratio of these counts \(N_A,0:N_A,90\) is equal to the ratio of \(c(K,90):c(K,0)\). Knowing the above ratio, one can obtain the \(K\) and \(c(K,0)\) values using the table provided by Mathieu and coworkers. Finally, it is easy to calculate the total capillary length knowing the \(J_A\) and the total volume of the tissue.

The results were statistically evaluated by analysis of variance (ANOVA) with subsequent Bonferroni test where applicable.

Results

Basic Data Including the Flow Measurements

Basic data are summarized in Table 1. Mean arterial pressure was significantly elevated in the renal hypertensive rats when compared with the sham-operated animals \((p<0.01)\), and no significant differences were detected between sedentary and trained rats. Similar results were obtained during the weekly systolic blood pressure measurements preceding the day the animals were killed. Chronic hypertension resulted in a significant increase in the left ventricular mass when compared with the normotensive animals \((p<0.01)\). These increases in the left ventricular mass of hypertensive rats were found in spite of a small, but significant, decrease in the body mass of these animals \((p<0.05)\).

Coronary blood flow and coronary resistance measurements on larger numbers of rats in the same
experimental groups and in a more detailed form have been published elsewhere. The key results for animals with detailed morphometric analysis of the coronary capillary bed are displayed in Table 1 to provide a basis for a comparison between function and structure of the coronary bed. The most important finding is a significant (p<0.001) increase in minimal left ventricular resistance per unit mass found in hypertensive animals, which is an indicator of a reduced coronary reserve. Exercise resulted in a decreased coronary reserve in sham-operated rats (p<0.05) but not in hypertensive animals.

### Myocardial Capillarization

Table 2 contains data concerning the myocardial capillary supply. All of these results were evaluated by a three-way analysis of variance (the effect of chronic hypertension, exercise, and sampling cardiac region). Capillary density was significantly lower (p<0.01) in the hypertrophic hearts from rats with renal hypertension when compared with normal hearts, but no significant effect of exercise or myocardial region was detected. The same results were found in the case of two parameters closely related to the capillary density: capillary domain and the radius of the tissue cylinder. Both were significantly increased in the hypertrophic hearts but were not influenced by chronic exercise or myocardial sampling region. Spacing of the capillary net apparently increased in hypertrophic hearts as a result of increasing cell size because the number of myocytes per capillaries was basically the same in all experimental groups. Variability of capillary spacing was characterized by the heterogeneity index log SD, which was significantly increased due to chronic hypertension (p<0.05) but not affected by chronic exercise or location within the ventricular wall (endomyocardium versus midwall).

Orientation of capillaries in space was evaluated by factors c(K,0) and K. Coefficient K was significantly lower in the hearts from renal hypertensive rats (p<0.01) when compared with the hearts from sham-operated animals and lower in the endomyocardium than in the midsection (p<0.05). On the other hand, no effects of exercise were detected. Consequently, the related correction factor c(K,0) was also significantly increased in hypertrophic hearts and in endomyocardium. Combination of these results with the capillary density yields the estimates of the capillary length density, i.e., capillary length per volume unit of myocardial tissue. Capillary length density was significantly decreased in the hypertrophic hearts from hypertensive rats (p<0.01), with no effects of chronic exercise or heart region. Finally, total capillary length per left ventricle, evaluated by a two-way analysis of variance, showed an increase in hypertrophic hearts (p<0.01) with no effect of swimming alone but a significant interaction between hypertension and swimming (p<0.05). The degree of increase in the total capillary length of hypertrophic hearts, however, is much less than the increase in the left ventricular weight as illustrated in Figure 1. Therefore, the capillary length density was significantly decreased in these hearts. On the other hand, a small increase in the left ventricular weight of sham-operated exercising animals was accompanied by a proportional increase in total capillary length as may be seen in Figure 1.

Flow and resistance measurements in the same animals in which the morphometric analysis of the capillary bed took place enabled us to compare these two sets of data. No functionally meaningful correlations were found between any of these indexes. While there was a weak, but significant, negative correlation between the minimal coronary resistance and the average capillary domain (p<0.05) this relation was obviously secondary due to a much higher degree of correlation of these two parameters with the left ventricular weight.

### Discussion

**Basic Data Including the Flow Measurements**

Chronic renal hypertension resulted in a sizeable increase in the left ventricular weight in the 40–50% range, which became even slightly higher when expressed as a relative left ventricular weight. This finding is in agreement with numerous reports on stimulated cardiac growth as a result of chronic pressure overload. Chronic exercise did not influence the degree of cardiac hypertrophy in the renal hyper-
Table 2. Various Indexes of Myocardial Capillarization

<table>
<thead>
<tr>
<th></th>
<th>Sham-Sed</th>
<th>Sham-Swim</th>
<th>RHR-Sed</th>
<th>RHR-Swim</th>
<th>Sign.</th>
</tr>
</thead>
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<tr>
<td>Capillary density No/mm²</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4,767 ± 165</td>
<td>4,592 ± 165</td>
<td>4,033 ± 127</td>
<td>3,995 ± 100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4,553 ± 157</td>
<td>4,780 ± 123</td>
<td>3,959 ± 158</td>
<td>3,994 ± 133</td>
<td></td>
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<tr>
<td>Capillary domain μm²</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>213 ± 7</td>
<td>221 ± 8</td>
<td>251 ± 8</td>
<td>252 ± 66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>223 ± 8</td>
<td>211 ± 6</td>
<td>258 ± 11</td>
<td>254 ± 9</td>
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<tr>
<td>Calculated radius of the tissue cylinder $R$ (μm)</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.2 ± 0.1</td>
<td>8.4 ± 0.1</td>
<td>8.9 ± 0.1</td>
<td>9.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Heterogeneity index log SD $R$</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.064 ± 0.02</td>
<td>0.062 ± 0.02</td>
<td>0.065 ± 0.02</td>
<td>0.065 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>No. of cells/capillaries</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.04 ± 0.01</td>
<td>1.04 ± 0.01</td>
<td>1.06 ± 0.01</td>
<td>1.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Concentration parameter $K$</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.30 ± 0.36</td>
<td>4.17 ± 0.44</td>
<td>4.01 ± 0.32</td>
<td>3.88 ± 0.16</td>
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<tr>
<td>Correction factor $c(K,0)$</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.34 ± 0.34</td>
<td>4.18 ± 0.17</td>
<td>3.61 ± 0.22</td>
<td>3.43 ± 0.24</td>
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<tr>
<td>Capillary length density mm/mm³</td>
<td>E</td>
<td>M</td>
<td>E</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5,186 ± 167</td>
<td>4,876 ± 153</td>
<td>4,541 ± 153</td>
<td>4,387 ± 102</td>
<td></td>
</tr>
<tr>
<td>Total capillary length/left ventricle</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td>m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,667 ± 67</td>
<td>2,998 ± 114</td>
<td>3,551 ± 160</td>
<td>3,374 ± 130</td>
<td></td>
</tr>
</tbody>
</table>

Mean values = SEM, 14 rats per group. Sham-operated and renal hypertensive rats (RHR) that were either sedentary (Sed) or swimming (Swim). E, endomyocardium; M, midmyocardium.

$p < 0.01$ and $p < 0.05$, significant effect of hypertension (three-way ANOVA); $p < 0.05$, significant effect of sampling region (three-way ANOVA).

tensive rats, while it produced a significant, albeit small (11%), increase in the left ventricular weight of sham-operated animals. These changes were accompanied by a decreased coronary reserve in hypertrophic hearts of both sedentary and exercising hypertensive rats. Similar decreases in coronary flow reserve were found in rats with both spontaneous and renal hypertension as well as in hearts from other experimental species exposed to chronic pressure overload. These data suggest that coronary vascular growth does not keep pace with myocardial hypertrophy.

Chronic exercise in sham-operated rats resulted in a moderate increase in coronary reserve. This improvement is in agreement with the majority of studies on the effect of chronic exercise, even though studies reporting unchanged coronary reserve can also be found. Apparently, the type and duration of chronic exercise, together with the animal species and age, may influence the final effect.

Our failure to detect favorable effect of exercise on coronary flow reserve in hypertrophic hearts is in agreement with the findings of Buttrick et al and Schaible et al. They reported that swimming can neither prevent nor reverse the decrement of coronary flow reserve to female hypertensive rats. These studies, however, were based on coronary blood flow measurements in isolated, retrograde perfused hearts. Our study confirms these results and extends them to the in vivo situation using conscious, unrestrained animals.

Myocardial Capillarization

Decreased capillary density, together with the unchanged myocyte-to-capillary ratio that we found in hypertrophic hearts of renal hypertensive rats, is in agreement with several similar studies on capillarization of hearts with chronic pressure overload (for a recent review, see Rakusan). Neither of these parameters was influenced by chronic exercise. The results demonstrate that physical exercise does not necessarily lead to an increased capillary supply of the heart as is often described in the literature. We recently compared various studies on capillary density in hearts from animals subjected to exercise and found three situations in which capillary density increased following various forms of chronic exercise, seven reports of no change, and four reports of decreased myocardial capillary density. The results demonstrate that physical exercise does not necessarily lead to an increased capillary supply of the heart as is often described in the literature. We recently compared various studies on capillary density in hearts from animals subjected to exercise and found three situations in which capillary density increased following various forms of chronic exercise, seven reports of no change, and four reports of decreased myocardial capillary density.
Capillary spacing was also relatively small, except in experimental aortic constriction. In rats with spontaneous hypertension, the increase in heterogeneity of capillary spacing, i.e., the variability estimated from the numerical capillary density on the tissue cross-sections. Coefficient K, which is an indicator of such an arrangement, was found to be significantly lower in the hearts from renal hypertensive rats, indicating less uniform arrangement around the axis of anisotropy. Consequently, the correction coefficient \( K \) is significantly higher in this experimental group, resulting in higher capillary length density than one would expect from the number of capillaries per unit area. This increase, however, is not high enough to compensate for the lower numerical capillary density in the hypertrophic heart, and the capillary length density remained significantly lower in hypertrophic hearts when compared with the hearts from normotensive animals. Once again, neither coefficient \( K \) nor \( c(K,0) \) were influenced by chronic exercise. Our estimates of the correction coefficient \( c(K,0) \), in the range of 1.07 to 1.09 for the normal hearts, are very similar to the \( c(K,0) \) of 1.065 found in normal hearts by Mattfeld and coworkers and 1.090 reported by Pietschmann and close to the values described for the feline soleus muscle. Chronic exercise did not influence significantly the degree of alignment in the coronary capillary bed in our experimental situation, which is in agreement with the results of Mattfeld et al. Significantly larger deviation from the ideal alignment with the axis of anisotropy found in hypertrophic hearts from the hypertensive rats in the present study is, to our knowledge, the first observation for hypertrophic hearts. Less homogenous alignment would also result in a greater heterogeneity of capillary spacing on the cross-sections. Thus, the heterogeneity index \( \log SD \) and anisotropy coefficients are interrelated.

Finally, the above parameters allowed us to also estimate the capillary length density and the total length of capillaries per left ventricle. Capillary length density was decreased in hypertrophic hearts when compared with normal hearts, even though the decrease was less than expected according to the numerical density of capillaries. This is due to greater length of the...
individual capillaries in the hypertrophic hearts [higher c(K,0)]. Once again, the effect of chronic exercise was not detectable either in normotensive or hypertensive rats. Mattfeld and coworkers\textsuperscript{23} reported a similar finding on the effect of chronic exercise in normotensive rats; however, we are not aware of a similar study on hypertensive animals. Knowing the capillary length density and the volume of the organ, it is also possible to calculate the total length of capillaries in that organ. We found a moderate, but significant, increase in the total length of coronary capillaries in the left ventricle of exercising rats from 2.7 km to 3.0 km. This increase is proportional to a similar increase in the ventricular weight as demonstrated in Figure 1. Further increases to 3.5 and 3.4 km were detected in sedentary hypertensive and exercising hypertensive rats, respectively. However, these increases were less than one would expect from the increase in the left ventricular weight, resulting in a less favorable capillary supply to hypertrophic hearts. In similar studies on the effect of a chronic running program on normotensive rats, Mattfeld and coworkers\textsuperscript{23} found an increase in the total capillary length commensurate with an increase in ventricular weight, while Kayar and coworkers\textsuperscript{27} reported an unchanged total capillary length in spite of increasing heart mass, resulting in a decreased capillary density. Our measurements in hypertrophic hearts of hypertensive rats are probably the first estimates of this type.

An increase in the total capillary length per left ventricle of hypertrophic hearts, when compared with hearts from sham-operated animals, is probably not due to a formation of new capillaries. The myocyte-to-capillary ratio remained unchanged in this experimental situation, and this form of cardiomegaly is characterized by a constant number of muscle cells. Thus, the total number of capillaries is also constant, and the length of the individual capillaries is increased in the hypertrophic hearts for the following reasons. First, capillary orientation is more irregular and capillaries are more tortuous in the hypertrophic hearts as indicated by a decreased concentration parameter and an increased correction factor (see Table 2). Secondly, cell length is also increased in hypertrophic hearts, and the capillaries merely follow the same number of muscle cells as in normal hearts.

Our morphometric observations were made on hearts from animals in which the coronary blood flow and coronary resistance were also measured. As mentioned in the results section, we found a weak, but significant, correlation between the minimal coronary resistance and various indexes of myocardial capillarization. The degree of correlation, however, is much higher between the minimal coronary resistance and left ventricular weight or between the indexes of myocardial capillarization and left ventricular weight. Thus, the coronary resistance is probably more dependent on the coronary arteries and arterioles than on the capillary bed.

In conclusion, we found that hypertrophic hearts from rats with renal hypertension have impaired capillarization. This is evident from the lower number of capillaries found in a unit of tissue cross-section. The arrangement of these capillaries is less regular than in normal hearts: the heterogeneity of capillary spacing on the cross-sections is increased, and the estimates of three-dimensional arrangement point toward less uniform arrangement around the preferential axis. Consequently, the length of the individual capillaries per unit of tissue volume is larger. This increase is, however, not sufficient to overcome the effect of reduced capillary numbers. In addition, the increased heterogeneity of capillary spacing impairs the geometrical conditions for oxygen supply. Chronic exercise does not alleviate any of these indexes of impaired capillarization in hypertrophic hearts. In the normotensive rats, chronic swimming results only in a moderate increase in the total capillary length associated with a similar, small increase in the left ventricular weight. Our results seem to indicate that a moderate increase in the myocardial capillary length can probably be induced by chronic exercise in normotensive rats. On the other hand, no beneficial effect was found from chronic exercise on capillarization of hypertrophic hearts from renal hypertensive rats.

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KEY WORDS  • hypertension  • terminal vascular bed  • oxygen supply to heart  • coronary reserve
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