Impaired Skin Capillary Recruitment in Essential Hypertension Is Caused by Both Functional and Structural Capillary Rarefaction

Erik H. Serné, Reinold O.B. Gans, Jan C. ter Maaten, Geert-Jan Tangelder, Ab J.M. Donker, Coen D.A. Stehouwer

Abstract—Capillary rarefaction occurs in many tissues in patients with essential hypertension and may contribute to an increased vascular resistance and impaired muscle metabolism. Rarefaction may be caused by a structural (anatomic) absence of capillaries, functional nonperfusion, or both. The aim of this study was to assess the extent of structural versus functional capillary rarefaction in the skin of subjects with essential hypertension. We examined skin capillary density with video microscopy before and during maximization of the number of perfused capillaries by venous congestion (structural capillary number) and before and during postocclusive reactive hyperemia (capillary recruitment, which may have a structural and/or functional basis). The study group was composed of 26 patients with never-treated essential hypertension and 26 normotensive control subjects. In both groups, intermittently perfused capillaries in the resting state were an important functional reserve for recruitment during postocclusive hyperemia. Recruitment of perfused capillaries during postocclusive reactive hyperemia was decreased in the hypertensive subjects compared with normotensive control subjects (47.9±6.8 versus 55.3±8.2 capillaries/mm², respectively; P<0.01). During venous occlusion, maximal capillary density was significantly lower in the hypertensive subjects than in the control subjects (52.5±6.6 versus 72.8±8.6 capillaries/mm², respectively; P<0.05), suggesting structural rarefaction. However, in the hypertensive subjects compared with the normotensive subjects, a smaller proportion of the maximal number of capillaries was perfused during postocclusive hyperemia (91.6±7.5% versus 97.2±2.7%, respectively; P<0.05), suggesting an additional functional impairment of capillary recruitment. If the difference in capillary numbers during venous congestion (≈4.6 capillaries/mm²) truly reflects the structural difference between the normotensive and hypertensive subjects, then, at most, 62% (4.6/7.4×100%) of the difference in capillary numbers during postocclusive hyperemia (≈7.4 capillaries/mm²) can be explained by structural defects, and at least 38% can be explained by functional defects. In conclusion, in patients with essential hypertension, recruitment of perfused capillaries is impaired, which can be explained by both functional and structural rarefaction. (Hypertension. 2001;38:238-242.)

Key Words: hypertension, essential ■ microcirculation ■ capillaries ■ vascular resistance

A decreased number of capillaries and arterioles, or so-called microvascular rarefaction, is a well-established abnormality that occurs in many tissues in patients with essential hypertension. Experimental and human studies suggest that this microvascular rarefaction contributes to an increase in vascular resistance and antedates the onset of sustained hypertension. In addition, decreased capillary density affects the spatial pattern of flow in the microvascular bed, causing a nonuniform distribution of blood flow among exchange vessels. Changes in capillary perfusion have been shown to influence skeletal muscle metabolism (ie, oxygen uptake and insulin-mediated glucose uptake) in the rat hindlimb. Hence, microvascular rarefaction, by affecting both pressure and flow patterns, may have consequences not only for peripheral vascular resistance but also for muscle perfusion and metabolism.

Direct intravital video microscopy, a dynamic method for studying skin capillaries, has recently been used to investigate whether capillary rarefaction in essential hypertension is caused by a structural (anatomic) absence of capillaries or by functional nonperfusion. Because this technique depends on the presence of erythrocytes inside capillaries for their identification, this method has the potential for missing nonperfused capillaries. To expose more nonperfused capillaries, both venous congestion and reactive hyperemia after arterial occlusion have been used. Whereas...
peak capillary density during venous congestion is thought to depend mainly on the anatomic number of capillaries,\textsuperscript{1, 15} postocclusive reactive hyperemia may be used to detect functional recruitment of initially nonperfused capillaries,\textsuperscript{11, 12, 15} which reflects both functional and structural factors.

By use of these techniques, a recent study demonstrated that both capillary density in the resting state and peak capillary density during venous congestion were significantly decreased in hypertensive subjects. In addition, postocclusive reactive hyperemia caused a decrease in the number of perfused capillaries rather than recruitment of nonperfused capillaries in both normotensive and hypertensive subjects.\textsuperscript{1} These findings led to the conclusion that the reduction in capillary density in hypertensive subjects was caused by a structural absence of capillaries. In contrast, we\textsuperscript{11, 12, 16} and others\textsuperscript{17} have observed recruitment of initially nonperfused capillaries during postocclusive reactive hyperemia in both normotensive and hypertensive subjects. In addition, capillary recruitment has been shown to be decreased in hypertensive subjects, whereas capillary density in the resting state was similar between normotensive and hypertensive subjects.\textsuperscript{12, 15} Moreover, in normotensive and hypertensive subjects, capillary recruitment during postocclusive reactive hyperemia was inversely associated with blood pressure.\textsuperscript{11, 12}

An explanation for these apparent discrepancies may be a methodological difference in defining capillary recruitment. Capillaries work on a “rota system”; i.e., some are temporarily perfused, whereas others are temporarily shut down,\textsuperscript{15} causing temporal heterogeneity in capillary perfusion. During direct intravital microscopy without dyes, the open time, i.e., the time that capillaries are filled with erythrocytes, varies greatly among different capillaries in the same visual field. Some capillaries seem to be continuously filled with erythrocytes, whereas others are intermittently perfused. In 1 study,\textsuperscript{1} capillary density in the resting state was determined by counting for 5 minutes to presumably detect all intermittently and continuously perfused capillaries; however, we only counted capillaries that were continuously perfused during a short (15-second) period,\textsuperscript{11, 12, 16} because intermittently perfused capillaries may be an important functional reserve that can be recruited during postocclusive reactive hyperemia.

The aim of the present study was to investigate whether methodological differences in determining capillary density in the resting state could explain these apparent discrepancies in the literature. In addition, we investigated the hypothesis that postocclusive reactive hyperemia may lead to an increase in the number of perfused capillaries (capillary recruitment) by prolonging the integrated open time of capillaries that are intermittently perfused in the resting state. Finally, we assessed the extent of structural versus functional capillary rarefaction in explaining the defects of capillary recruitment in the skin of subjects with essential hypertension.

Methods

Subjects
Twenty-six nondiabetic subjects with never-treated essential hypertension and 26 normotensive healthy control subjects matched for age and gender participated in the present study (Table 1). All were white and nonsmokers. The inclusion criteria for the hypertensive subjects were as follows: blood pressure as determined by triplicate office measurement >140/90 mm Hg and (for ethical reasons) <180/110 mm Hg, age 30 to 70 years, normal fasting glucose according to the criteria of the American Diabetes Association,\textsuperscript{18} and no signs or symptoms of cardiovascular or other concomitant disease. Secondary forms of hypertension were excluded by medical history and standard laboratory tests. The study protocol was approved by the local ethics committee, and informed consent was obtained from each subject.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hypertensive Subjects</th>
<th>Normotensive Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, n</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Male, n</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age, y</td>
<td>49.1 ± 10.8</td>
<td>49.1 ± 10.4</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>27.9 ± 4.9</td>
<td>25.1 ± 2.9*</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>155 ± 9.6</td>
<td>128 ± 6.8†</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>101 ± 8.3</td>
<td>80 ± 6.9†</td>
</tr>
<tr>
<td>Heart rate during daytime, bpm</td>
<td>76 ± 11</td>
<td>76 ± 7.9</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>5.0 ± 0.5</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Fasting serum total cholesterol, mmol/L</td>
<td>5.2 ± 0.8</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>Fasting HDL cholesterol, mmol/L</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Fasting serum triglycerides, mmol/L</td>
<td>1.4 ± 0.7</td>
<td>1.2 ± 0.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD. SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

*P < 0.05 and †P < 0.001 for hypertensive vs normotensive subjects.

Intravital Capillaroscopy

The capillaroscopy studies were conducted in a standardized manner by using capillary microscopy equipment as previously described in more detail.\textsuperscript{11, 12, 16}

Reactive hyperemia after 4 minutes of arterial occlusion was used to assess functional recruitment of capillaries.\textsuperscript{11, 12, 16, 17} A visual field of 1 mm\(^2\) was recorded before and after arterial occlusion with a digital cuff (Digit cuff, Hokanson), and the images were stored on videotape. The number of capillaries was counted offline by a single experienced investigator (E.H.S.) from a freeze-framed reproduction of the videotape and from the running videotape if it was uncertain whether a capillary was present or not. The investigator counting the capillaries was blinded to the hypertensive status and blood pressure data of the study subjects. Capillary density in the resting state was counted during a 15-second period and a 3-minute period. During the 15-second period, only continuously perfused capillaries were counted, although intermittently perfused capillaries were also visible. During the 3-minute period, all (continuously and intermittently) perfused capillaries were counted. Directly after release of the cuff, the number of continuously perfused capillaries was counted. (The increase in capillary number occurs within a few seconds.) Capillary density was defined as the number of erythrocyte-perfused capillaries per square millimeter of nailfold skin. This procedure was then repeated by using a visual field adjacent to the first visual field. Data concerning capillary densities are the mean of these 2 measurements. Percentage capillary recruitment was assessed by dividing the increase in capillary density during postocclusive reactive hyperemia by the capillary density in the resting state. (Note that this procedure yields 2 different estimates of percentage capillary recruitment, depending on whether capillary density in the resting state is defined by counting for 15 seconds or for 3 minutes.) The day-to-day coefficient of variation of percentage capillary recruitment during
Capillaroscopy Data in Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Capillaroscopy Data</th>
<th>Hypertensive Subjects</th>
<th>Normotensive Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>During reactive hyperemia after arterial occlusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin temperature, °C</td>
<td>31.3±0.9</td>
<td>31.5±0.9</td>
</tr>
<tr>
<td>Capillary density in the resting state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-s count,* capillaries/mm²</td>
<td>39.3±5.0</td>
<td>38.8±4.7</td>
</tr>
<tr>
<td>3-min count,† capillaries/mm²</td>
<td>45.8±4.9‡ $</td>
<td>52.6±5.7§</td>
</tr>
<tr>
<td>Peak capillary density, capillaries/mm²</td>
<td>47.9±6.8‡ $</td>
<td>55.3±8.2</td>
</tr>
<tr>
<td>Absolute increase from the resting state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-s count,* capillaries/mm²</td>
<td>8.5±2.4¶</td>
<td>16.7±4.9</td>
</tr>
<tr>
<td>3-min count,† capillaries/mm²</td>
<td>2.0±2.5</td>
<td>2.7±3.4</td>
</tr>
<tr>
<td>Capillary recruitment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-s count, † %</td>
<td>21.7±5.8¶</td>
<td>43.1±10.9</td>
</tr>
<tr>
<td>3-min count, † %</td>
<td>4.2±5.2</td>
<td>4.8±5.9</td>
</tr>
<tr>
<td>During venous congestion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin temperature, °C</td>
<td>31.2±0.9</td>
<td>31.2±0.9</td>
</tr>
<tr>
<td>Peak capillary density, capillaries/mm²</td>
<td>52.5±6.6¶ $</td>
<td>57.2±8.6**</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Continuously perfused capillaries.
†Continuously plus intermittently perfused capillaries.
‡P<0.05 for hypertensive vs normotensive subjects after adjustment for body mass index; §P<0.001 for 15-s count vs 3-min count; and ¶P<0.01 and **P<0.001 for venous occlusion vs postocclusive reactive hyperemia.

Postocclusive reactive hyperemia was 8.3±4.9%, as determined in 9 subjects on 2 occasions.

Fifteen minutes after performing the postocclusive reactive hyperemia procedure, we applied venous congestion, with the digital cuff inflated to 60 mm Hg for 60 seconds, to expose a maximal number of nonperfused capillaries. Using the same visual fields that were used during postocclusive reactive hyperemia, the capillaries were counted in the 60-second recordings. The day-to-day coefficient of variation of peak capillary density during venous congestion was 9.5±7.1%, as determined in 9 subjects on 2 occasions. Venous congestion at 60 mm Hg for 120 instead of 60 seconds did not increase the number of visible capillaries further (determined in 9 healthy subjects, data not shown).

Statistical Analysis

Data are expressed as mean±SD, unless stated otherwise. Comparison of normotensive and hypertensive subjects was performed with the Student’s t test or a nonparametric variant in case of a nonnormal distribution of the variables. MANOVA was used to compare microvascular measurements between the groups after adjustment for differences in body mass index. A paired Student’s t test was used to compare capillary densities before and during postocclusive reactive hyperemia and venous congestion. A 2-tailed value of P<0.05 was considered significant. All analyses were performed on a personal computer using the statistical software package SPSS, version 9.0.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Table 2 and the Figure show the capillaroscopy data. A significantly larger number of perfused capillaries could be detected when capillary density in the resting state was counted for 3 minutes instead of only 15 seconds (P<0.001 for both groups). Both postocclusive reactive hyperemia and venous congestion increased the number of perfused capillaries. The increase in capillary density from the resting state with reactive hyperemia was significant in both the normotensive (16.7±4.9 capillaries/mm², P<0.0001) and the hypertensive (8.5±2.4 capillaries/mm², P<0.0001) subjects when the 15-second baseline count was used. When the 3-minute baseline count was used, the increase in capillary density was also significant in both the normotensive (2.7±3.4 capillaries/mm², P<0.001) and the hypertensive (2.0±2.5 capillaries/mm², P<0.001) subjects. From the video image, it was obvious that part of the perfused capillaries visible during postocclusive reactive hyperemia, directly after release of the arterial occlusion, consisted of capillaries that were intermittently perfused in the resting state.

Venous congestion was associated with a significantly greater capillary number than was postocclusive reactive hyperemia in both the hypertensive subjects (P<0.01) and the normotensive subjects (P<0.05). The number of capillaries perfused during postocclusive reactive hyperemia (expressed as a percentage of the maximal capillary number after venous congestion) was significantly lower in the hypertensive than in the normotensive subjects (91.6±7.5% versus 97.2±2.7%, respectively; P<0.05). The number of capillaries perfused in the resting state (counted for 3 minutes and expressed as a percentage of the maximal capillary number after venous congestion) was also significantly lower in the hypertensive than in the normotensive subjects (87.9±6.5% versus 93.1±2.6%, respectively; P<0.05). If the difference in capillary numbers during venous congestion (≈4.6±8.6 capillaries/mm², Table 2) truly reflects the structural difference between the normotensive and hypertensive subjects, then at most ≈62% (4.6/7.4×100%) of the difference in capillary numbers during postocclusive hyperemia (≈7.4±8.1 capillaries/mm², Table 2) can be explained by structural defects, and at least 38% can be explained by functional defects.

Discussion

A reduction in the density of capillaries (rarefaction) has been demonstrated in many tissues in patients with essential
hypertension.1–4 This rarefaction, by affecting both pressure and flow patterns, may have consequences for peripheral vascular resistance1,11,12,16 and for muscle perfusion and metabolism.10–12

The present study demonstrates that the number of capillaries perfused in the resting state was reduced in the hypertensive subjects compared with the normotensive subjects when both continuously and intermittently perfused capillaries were counted for 3 minutes. No difference in capillary density could be detected when only the number of capillaries continuously perfused in the resting state was counted for 15 seconds. In the hypertensive subjects, peak capillary density during postocclusive reactive hyperemia was decreased. During postocclusive reactive hyperemia, a substantial part of the capillaries perfused directly after release of the arterial occlusion consisted of capillaries that were intermittently perfused in the resting state. Therefore, capillaries intermittently perfused in the resting state seem to be an important functional reserve that can be recruited during postocclusive reactive hyperemia. The present study confirms previous findings that the maximal number of capillaries seen with venous congestion exceeds that seen with postocclusive reactive hyperemia and is lower in hypertensive subjects, suggesting a structural reduction in capillary density.1,15 In the hypertensive subjects compared with the normotensive subjects, however, a smaller proportion of the maximal number of capillaries was perfused in the resting state and during postocclusive reactive hyperemia, thus suggesting an additional functional impairment of capillary perfusion and capillary recruitment in these patients.

Our results strongly suggest that the discrepant findings from earlier studies1,11,12,15–17 can be explained by the different definitions of capillary density in the resting state and during postocclusive reactive hyperemia used in these studies. From the video image, it was obvious that part of the perfused capillaries visible during postocclusive reactive hyperemia, directly after release of the arterial occlusion, consisted of capillaries that were intermittently perfused in the resting state. This suggests that capillaries that are intermittently perfused in the resting state may be an important reserve that can be recruited during postocclusive reactive hyperemia. Therefore, the reduced number of capillaries in the resting state during the 3-minute count and during postocclusive reactive hyperemia in the hypertensive subjects may be due to a decreased number of intermittently perfused capillaries. These abnormalities do not simply reflect the functional consequences of a structural absence of capillaries, because even though the maximal number of capillaries in the hypertensive subjects, compared with the normotensive subjects, should have been sufficient to allow the same absolute or relative numbers of capillaries to be perfused in the resting state, the hypertensive subjects demonstrated a reduced absolute and relative (absolute number expressed as a percentage of maximal skin capillary density) number of perfused capillaries in the resting state. Likewise, peak capillary density during postocclusive reactive hyperemia (expressed as a percentage of maximal skin capillary density) was lower in the hypertensive subjects, although again the maximal number of capillaries should have allowed the absolute and relative increase in capillary number during postocclusive reactive hyperemia to be similar in both groups. These data are consistent with a true functional impairment of capillary perfusion and capillary recruitment in the hypertensive subjects. If the difference in capillary numbers during venous congestion truly reflects the structural difference between the normotensive and hypertensive subjects, then at least 38% of the difference in capillary numbers observed during postocclusive hyperemia can be explained by functional defects.

The mechanisms that are responsible for the functional impairment of capillary recruitment during postocclusive reactive hyperemia in essential hypertension remain elusive. Postocclusive reactive hyperemia that is used to induce capillary recruitment is thought to be determined at the level of the small arterioles19 and to be independent of the autonomic nervous system.20,21 The myogenic response to a change in intravascular pressure is postulated to be the prime stimulus determining peak hyperemia, whereas local metabolic factors act in a synergistic role to prolong the response.21–23 An occlusion of 4 minutes, as applied in the present study, is thus expected to cause vasodilatation not only through the mechanism of myogenic vasodilatation but also through an additional metabolic vasodilatory stimulus.17,24 The role of the endothelium in modulating the myogenic response is controversial,25 but in hypertension, arteriolar vasomotor responses to changes in intraluminal pressure are due to at least 2 mechanisms: one that is intrinsic to vascular smooth muscle (ie, myogenic) and a second that involves the endothelium.26 Therefore, an increased sensitiv-
In conclusion, the present study demonstrates that capillaries that are intermittently perfused in the resting state are an important reserve that can be recruited during postocclusive reactive hyperemia. In essential hypertension, recruitment of perfused capillaries is impaired, which can be explained by functional and structural rarefaction for \( \approx 38\% \) and \( \approx 62\% \), respectively.

Acknowledgments
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References
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