Attenuation of the Microcirculation in Young Patients with High-Output Borderline Hypertension

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SUMMARY Previous studies have shown abnormalities of the microvasculature in the spontaneously hypertensive rat and human subjects with established hypertension. We have studied the conjunctival microvasculature in relation to systemic and forearm hemodynamics in 24 normal subjects (NL) and 10 subjects with intermittent elevation of blood pressure (BHT). Macrophotographs of the conjunctival circulation were measured for arteriolar diameter and density of arterioles, capillaries, and venules. Blood pressure was measured by Arteriosonde, cardiac index by echocardiography, and forearm hemodynamics by mercury-filled strain-gauge venous occlusion plethysmography. Average diastolic blood pressure in the NL group was 74 ± 1.7 mm Hg, while that of the BHT subjects was 89 ± 3.1 mm Hg (p < 0.005). Capillary density, venous density, and total vascular density were significantly lower in the BHT than NL group, while arteriolar density did not differ significantly. Cardiac index was significantly higher, and peripheral vascular resistance significantly lower, in the BHT as compared to the NL subjects. Forearm blood flow was higher in the NL subjects. The diameter of the preterminal arterioles of the BHT subjects was 27% greater than NL (p < 0.02). The capillary density was inversely related to the cardiac index (r = -0.482, p < 0.01), but was not related to blood pressure (r = -0.207). We conclude that the high cardiac output phase of early essential hypertension in humans is accompanied by a reduction in the number of filtering capillaries, and that the rarefaction of capillaries is more closely related to the elevation of cardiac output than to raised blood pressure. (Hypertension 5: 844-851, 1983)

KEY WORDS • hypertension • microcirculation • hemodynamics • echocardiography • plethysmography

For the past several decades, attention has been focused on the microvasculature of hypertensive subjects and experimental animals in an attempt to uncover the reason for the elevated total peripheral resistance that underlies most established forms of hypertension. Earlier studies in human subjects with moderate to severe hypertension have revealed capillary^1,2 and arteriolar3,5 narrowing, and a reduction in the number of arterioles in the conjunctiva.3 Studies in skeletal muscle of the spontaneously hypertensive rat (SHR) have shown a rarefaction of arterioles and capillaries,6,7 which in the gracilis muscle proceeds from a stage of capillary rarefaction at 6-8 weeks of age, through a stage of functional arteriolar rarefaction at 12-14 weeks of age, followed by a true anatomical rarefaction of arterioles at 16-18 weeks of age.8 At the early stages of hypertension, the SHR arterioles show no reduction in diameter compared to normotensive Wistar-Kyoto rats (WKY),9,10 but by 12-14 weeks of age there is pronounced arteriolar constriction.9

The objectives of our study were to determine whether the early microvascular changes described in the SHR were present in human borderline hypertension, since previous studies have been focused on individuals with fixed, well-established hypertension, and therefore more likely to uncover secondary changes; and to determine whether the changes in microvasculature were related to abnormalities of systemic or forearm hemodynamics, which are known to be present as hypertension develops in humans.

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Methods

The protocol for this study was approved by the Institutional Review Board of the University of Tennessee Center for the Health Sciences. All participants gave their informed consent.

The subjects of our study included 24 normal individuals and 10 with borderline hypertension, i.e., diastolic pressures above 90 on three occasions and beneath 90 the rest of the time (table 1). The average age of the normal subjects was 26.4 years, that of the hypertensives, 33.8 years. The hypertensive subjects tended to be somewhat heavier than the normal subjects. The differences in ages and weights were not statistically significant. Roughly half of each group were men and most were white. A positive family history of hypertension was present in 15 of 24 normal subjects and 9 of 10 borderline hypertensive subjects.

All subjects were admitted to the Clinical Research Center of the University of Tennessee for interview, physical examination, and clinical laboratory studies that consisted of a complete blood count, urinalysis, serum creatinine, endogenous creatinine clearance, blood sugar and serum sodium, potassium, chloride, and bicarbonate determinations. Blood pressure was measured in triplicate before each meal with an Arteriosonde after 5 minutes of rest in the supine position. Plasma renin activity and 24-hour urinary excretion of sodium, potassium, and creatinine were measured on the same day. Plasma renin activity was measured by radioimmunoassay with incubation at pH 5.7. Urinary sodium and potassium concentrations were measured on the first day of the study while the patients followed their usual diet. Hemodynamic studies were performed on the same day. Plasma renin activity was measured by radioimmunoassay with incubation at pH 5.7. Urinary sodium and potassium concentrations were measured with a flame photometer. The hemodynamic studies included echocardiography, from which stroke volume, cardiac output, and total peripheral resistance were determined, and venous occlusion plethysmography, from which forearm blood flow, forearm vascular resistance, venous compliance, and capillary filtration coefficient were calculated.

Echocardiographic Studies

Echocardiographic studies were performed with the patients lying on the left side. Images were obtained using Toshiba SSH-10 phased-array, real time, and combined M-mode ultrasonography (Toshiba Corporation, Tokyo, Japan). This instrument displays the M-mode marker as well as the real-time cardiac image. The transducer was placed on the chest wall and oriented to display the long axis view, which represents a sagittal cut through the heart from left ventricular apex to aortic root. The M-mode cursor was then positioned to intersect the maximum diameter of the left ventricle below the mitral valve leaflets and superior to the papillary muscles. This added step assures that the area selected reflects mid-wall motion and that the same dimensions are identified on subsequent studies. The motion mode ultrasound information was then recorded on a Toshiba strip chart recorder at 25 and 50 mm/sec through three or four respiratory cycles with the patient breathing quietly. Auscultatory pressures were taken at the same time and recorded.

The records were then measured using conventional measuring sites for left ventricular end diastolic dimension and end systolic dimensions. Systolic and diastolic volumes were calculated using the regression equation derived by Meyer et al.: left ventricle = \(-19.1 + 14.6 \text{Be} + 0.62 \text{Be}^2\) with Be representing the echo-determined left ventricular dimension. Meyer and his coworkers correlated their echocardiographic data with biplane angiograms to obtain regression analysis for small to normal adult-sized left ventricles.

Stroke volume was determined by subtracting the systolic from diastolic volumes. Cardiac output was calculated as the product of simultaneous heart rate and stroke volume averaged over 10 seconds. Mean arterial blood pressure equaled the sum of the diastolic pressure plus one-third of the pulse pressure. Total peripheral resistance calculated from the Frank formula using auscultatory blood pressure measurements simultaneous with the echocardiographic studies:

\[ R(\text{dyne cm}^{-1} \text{sec}) = \frac{\text{mean arterial pressure (mm Hg)} \times 1330}{\text{cardiac output (ml per sec)}} \]

In separate experiments we analyzed our method for reproducibility. Fifty-three subjects with normal ventricles were studied on two separate occasions after 30 minutes of rest in the supine position. At the time of each study, triplicate measurements were made over a 20-minute period. The average variation of cardiac outputs calculated by this method was 11.6%. The accuracy and reproducibility of echocardiographic measurements of left ventricular volume in subjects with normal hearts have been confirmed in several studies.

Forearm Hemodynamic Studies

Plethysmography monitors changes in forearm volume with a mercury-in-Silastic strain gauge secured to the forearm of the subject. This technique is employed to measure: forearm blood flow, capillary filtration coefficient, and venous capacitance. The mercury-filled, single-strand strain gauge was connected to an impedance-matching circuit, a high-sensitivity, alternating current, carrier preamplifier, and an 8-channel Hewlett-Packard 7788A recorder (Hewlett-Packard Corporation, Waltham, Massachusetts). The strain gauge was activated by a constant current. Small
changes in forearm circumference caused changes in the length of the strain gauge which resulted in linear changes in voltage across the ends of the strain gauge which were displayed on the recorder. Patients were examined, while supine, after 10 minutes of rest. Arterial blood pressure was measured by auscultation at the time the plethysmographic tracing was made. The strain gauge-to-pen deflection ratio was determined by dividing the stretch distance of the strain gauge by the pen deflection. This calibration constant was measured for each patient. The strain gauge was wrapped around the left forearm approximately 10 cm below the elbow, stretched 2% past its resting length and secured with tape. A blood pressure cuff was placed on the upper left arm and inflated to 40 mm Hg to block venous return at the time of the tracing. Three resting tracings were made while simultaneously taking a blood pressure reading on the right arm.

The initial slope of plethysmographic record results from the veins filling with blood since venous return is blocked by the cuff inflated to 40 mm Hg. The forearm blood flow was calculated from this first slope by the formula:

\[
\text{FBF (ml/min/100 g)} = \frac{2(1\text{st slope})(\text{calibration ratio})(\text{chart speed mm/min})(100)}{\text{forearm circumference (L) mm}}
\]

As the veins become fully distended, a second slope is recorded as fluid leaves the capillaries. Capillary filtration coefficient (ml/min/mm Hg/100 g) was calculated from the second slope using the formula:

\[
\text{CFC} = \frac{2(2\text{nd slope})(\text{calibration ratio})(\text{chart speed mm/min})(1 + \frac{Rv}{Ra})(100)}{40 \times (L) \text{ mm}}
\]

If a tangent is drawn from the second slope back to an erect perpendicular line drawn at the time the cuff was inflated, then the height of the line where it meets the tangent represents the venous capacitance at 40 mm Hg. Venous capacitance was calculated from the formula:

\[
\text{VC (ml/100 g)} = \frac{2(2\text{nd slope})(\text{calibration ratio})(100)}{L \text{ mm}}
\]

An automatic blood pressure reading was taken during each tracing and mean arterial pressure was calculated: \( S - D/3 + D \). By using mean arterial pressure and the calculated forearm blood flow, forearm vascular resistance is obtained:

\[
\text{FVR} = \frac{\text{MAP}}{\text{FBF}}, \text{ with units expressed as mm Hg/ml/min/100 g.}
\]

**Conjunctival Macrophotography**

After 10 minutes of rest in a temperature-controlled room, photographs were made with a Nikon F-2 camera fitted with a modified microscope lens and strobe lighting using Kodak Technical Pan 2415 film. The negative contained a five-fold magnification, which was enlarged to 50 times life size (fig. 1).

**Stereology of Conjunctiva Photographs**

All measurements were made without knowledge of the subject's medical history or blood pressure. The arterioles were overdrawn in red and the venules in black. Due to the curvature of the eye, the entire photograph was not always in focus. If such was the case, the area in focus was outlined in green. The photograph was then covered with a 10 \( \times \) 10 square grid overlay. Each square had a point in the center and the number of points covering the area in focus was counted. The length of the grid covering the area of interest (L) is then: \( L = 2 \times Pmd \), where \( Pm \) is the number of points over the area, and d is the width of one square with respect to the microcirculation. In these experiments the width of the square was 293.2 \( \mu \)m. The intersections of the arterioles, capillaries, and venules with the grid were determined individually. Out of focus vessels from underlying tissue often appear on the photograph and were not included in these measurements; out of focus vessels were usually large venules without sharp, clear edges and thus were easily distinguished from vessels in the surface layers of the conjunctiva. The length per unit area (Lc) for each vessel type was then calculated as:

\[
\frac{Lc = \pi \times Nc}{2 \times L},
\]

where \( Nc \) is the number of intersections. Measurements from all the photographs of a single subject were averaged to obtain one value.

The diameters of the arterioles were measured from the photographs with dial-indicator metric calipers (Rolyyn Optics, Arcadia, California) reading to 0.05 mm (1 \( \mu \)m with respect to the microcirculation). The arterioles were grouped under three orders of branching. The arterioles giving rise to the capillaries were called terminal arterioles, the ones giving rise to terminal arterioles were called preterminal arterioles and the vessels from which they arose were called large arterioles. All arterioles of each classification from a single subject were averaged so that "n" always refers to the number of subjects.

**Data Handling**

All data were tabulated in original and translated forms and entered on punch cards. Data were tested for significance by analysis of variance taking into account repeated measures using a PDP 11/70 computer (Digital Equipment Corporation, Maynard, Massachusetts). A Newman-Keul's a posteriori test was used to determine where differences lay when significant differences were found during analysis of variance. The significance of differences in distribution of vessels was analyzed by Fisher's exact test.

**Results**

The hemodynamic results are shown in table 2. The means of nine measurements of systolic and diastolic blood pressure made on the day of the study were significantly higher in the borderline hypertensive sub-
FIGURE 1. Fifty-fold magnification of lateral bulbar conjunctival vessels of a normal subject (A) and a patient with borderline hypertension (B). Examples of arterioles are indicated by (a), venules by (v), and capillaries by (c).
subjects. Similarly, cardiac index was significantly higher in these borderline hypertensive individuals. Total peripheral resistance was significantly lower. Forearm blood flow was also significantly lower. There was no significant difference in forearm vascular resistance, capillary filtration coefficient, venous capacitance, plasma renin activity, or urinary sodium excretion.

Figure 2 shows the conjunctival vessel lengths per unit area in the normal and in the hypertensive subjects. There was a significant reduction in the total length of capillaries per unit area (density) in the individuals with borderline hypertension (5.43 ± 0.38 vs 3.31 ± 0.63 mm/mm², p < 0.01), similar to findings in the SHR. There were significantly fewer venules (3.29 ± 0.21 vs 2.49 ± 0.30 mm/mm², p < 0.05), and a significant decrease in the total vascular density (9.77 ± 0.55 vs 6.52 ± 0.96 mm/mm², p < 0.01) in the borderline hypertensive subjects. The differences in arteriolar density were not significant (1.10 ± 0.09 vs 0.85 ± 0.15 mm/mm²).

The relationships between the cardiac index and the capillary density in both the normal and the hypertensive subjects are shown in Figure 3. A significant inverse correlation was found between cardiac index and capillary density. The higher the cardiac index, the greater the attenuation of the capillary bed.

Figure 4 displays the conjunctival arteriolar diameters of the normal and borderline hypertensive subjects. This classification, according to branching, was adapted from one used in the cremaster muscle except that we worked from the capillary up the vascular tree. The vessels do not show as great a decrease in diameter as those in the cremaster, but the conjunctiva has less metabolic demands than the vessels of skeletal muscle and its vessels probably do not carry as much blood flow. Functionally, however, the vessels are different according to the extent of tissue they supply. The terminal arterioles control flow to only a few capillaries, but the larger arterioles control flow to a large area of tissue. The arteriolar diameters of the normal subjects showed the usual decrease in diameter with subsequent branching. However, we observed an increased diameter of the preterminal arterioles (23.2 ± 0.85 vs 29.5 ± 2.4 μ, p < 0.02) of the hypertensive subjects, which was statistically significant. The mean terminal arteriolar diameter of the normal subjects was 18.4 ± 0.76, which did not differ significantly from that of the borderline hypertensives. 21.8 ± 1.71 μ. Fewer large arterioles appeared in the photographs of hypertensive subjects than in those of normal subjects. By Fisher's exact test, this difference in relative distribution did not reach statistical significance (p = 0.07).

**Discussion**

In the present study of borderline hypertensive subjects, the microcirculatory changes were not striking to the eye. It was not possible to distinguish a conjunctival photograph of a hypertensive subject from a normotensive one without the stereological measurements. The microcirculatory changes were similar to the findings in the SHR, which have capillary rarefaction and no reduction in arteriolar diameter in the early stages. One possible explanation of these microcirculatory alterations is that a rising cardiac output has two effects. First, an increase in blood pressure which stimulates the baroreceptors resulting in reduced sympathetic tone to the arterioles. Second, the smallest arterioles, which control the capillary density, also respond to local regulatory mechanisms. In an attempt to autoregulate blood flow, their tone may increase, resulting in a reduction in capillary density. In these early stages the increased tone in the terminal
portion of the arterioles is difficult to determine while the lack of enhanced tone upstream is readily observed.

When the baroreceptors adapt and sympathetic tone increases one would expect vasoconstriction in the upstream arterioles to become apparent. As blood pressure rises in the SHR, vasoconstriction increases and a large portion of the smaller arterioles are closed to flow under resting conditions, but they can be opened with sodium nitroprusside. This state of functional rarefaction of arterioles then progresses to one of anatomical rarefaction as the hypertension becomes fully developed. In the human conjunctiva a similar progression may take place resulting in the arteriolar rarefaction and vasoconstriction seen by Harper et al. in established hypertension.

Although not statistically significant, the trend toward lower capillary filtration coefficients and venous capacitance in the forearm (table 2) would be what one would expect to find in the conjunctiva if it could be measured. The capillary filtration coefficient includes surface area and permeability and a reduction in capillary and venular density would certainly give a reduced surface area of exchange (fig. 2). This reduction in capillary and venular density is consistent with the results reported by Edwards and Diana showing a decrease in capillary filtration coefficient in the hindquarters of the SHR. It also suggests that the microvascular alterations in the conjunctiva are not unique and the results are indicative of microvascular changes in other parts of the body.

Another area of similarity between borderline hypertensive subjects and the SHR is the lack of a contribution of increased wall/lumen ratio to arteriolar diameter reduction. According to the hypothesis proposed by Folkow et al., medial hypertrophy of precapillary resistance vessels encroaches upon the lumen resulting in a morphological increase in resistance. However, in SHR skeletal muscle, the arterioles do not show any increase in wall/lumen ratio. Vascular wall thickness could not be measured in the present study, but there was no reduction in arteriolar lumen size, so that even if hypertrophy occurred it did not result in a morphological resistance increase.

The objectives of our study were to determine whether the microvascular changes described in the young SHR were present in human borderline hypertension, as previous studies have focused on individuals with moderate to severe, chronic hypertension, and to determine whether these changes are related to abnormalities of peripheral or systemic hemodynamics.

We found that in comparison with normal subjects, individuals with borderline hypertension have a higher resting blood pressure, a higher cardiac index, lower total peripheral vascular resistance, lower forearm blood flow, a reduced length of conjunctival capillaries and venules per unit area, and conjunctival preterminal arterioles of greater diameter. A significant inverse relationship was found between cardiac index and conjunctival capillary length in normal and border-
line hypertensive subjects, which is consistent either with autoregulation taking place in this particular tissue in response to the elevated flow or with fewer capillaries from birth; this might necessitate increased flow to maintain normal capillary exchange of nutrients and waste products. Additional studies would be required to clarify this point. However, the studies of Henrich et al. demonstrating no differences in the single capillary surface areas of normal and spontaneously hypertensive rats, despite reduced numbers in the SHR, suggest that the rarefaction of capillaries has occurred in response to the elevated cardiac output in order to normalize exchange functions. The findings of the human conjunctiva reported herein have many similarities to the findings in the early stages of hypertension (4–8 weeks of age) in the SHR.

Individuals whose resting blood pressure transiently rises above 140/90 mm Hg but is normal the rest of the time are designated labile or borderline hypertensives. Widimsky et al. found that as a group, these individuals had an elevated cardiac output at rest, a total peripheral resistance that fell within the normal range, and an elevated blood pressure secondary to the increase in cardiac output. Eich and his coworkers studied an older group with labile hypertension and also found an elevated cardiac output with a vascular resistance within the normal range. However, Julius and his colleagues have found that a spectrum of hemodynamic changes was present in individuals with labile hypertension ranging from a very high cardiac output to one that was relatively reduced.

The increased cardiac output could be explained by enhanced activity of the autonomic nervous system. The evidence in favor of this postulate is the fact that such individuals have an increased heart rate and an increased left ventricular ejection rate. Julius et al. studied the effect of beta-adrenergic and parasympathetic inhibition in young patients with labile hypertension and found that this dual blockade resulted in a return of cardiac output to normal levels. They also studied the effect of interventions, such as exercise and infusion of dextran, and found that although peripheral vascular resistance was ordinarily within normal range at rest, resistance during any hemodynamic intervention was higher in the labile hypertensives than in the normotensive individuals.

Similarly, Sannerstedt and his coworkers studied the degree to which cardiac output and vascular resistance change with exercise in patients with labile hypertension and have found that the slope of the line relating output to resistance is actually inappropriately elevated in individuals with mild labile hypertension. Body fluid compartments have also been examined in an attempt to find the reason for the elevation of cardiac output. Ulrych et al. found that intravascular volume appeared to be redistributed toward the central circulation in patients with labile hypertension, thus increasing return to the right heart and cardiac output via the Starling mechanism.

It has also been proposed that the kidneys of hypertension-prone individuals are unable to excrete a dietary sodium load unless the renal perfusion pressure is raised above usual limits. Thus, a genetically susceptible individual, exposed to high environmental dietary sodium, would develop salt and water retention, expansion of plasma volume, increase in cardiac output, autoregulation would occur, peripheral vascular resistance would rise, and hypertension would develop.

Thus, patients with labile, borderline hypertension are characterized by an increased cardiac output, a central redistribution of blood volume, and evidence of enhanced activity of the autonomic nervous system leading to an increased heart rate, an increase in left ventricular ejection rate and an inability to drop forearm vascular resistance adequately in response to circumstances demanding an increase in blood flow.

In studies of the microcirculation, Lack observed that patients with diastolic pressures above 100 mm Hg commonly have narrowed, elongated, tortuous capillaries with sharp angular turns, thick walls, and impaired distensibility. Lee and Holze reported that subjects with diastolic pressures above 100 have metarteriolar narrowing, increased vasomotor activity and coiling, looping, and tortuosity of many vessels. Landau and Davis described marked thinning of capillaries in approximately half the hypertensive subjects in their study. Short reported changes in the intestinal arterioles of severely hypertensive subjects coming to autopsy. These individuals, whose diastolic blood pressures were greater than 120 mm Hg, had an increased wall-to-lumen ratio, no increase in the cross-sectional area of their arterial walls and a constricted arteriolar diameter. Harper and his colleagues observed a 20% reduction in arteriolar density and a 5% reduction in arteriolar diameter in the conjunctiva of human hypertensive subjects. Our studies demonstrate that attenuation of the microcirculation is present in even milder forms of hypertension than those reported previously and is related to the increase in cardiac index.

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