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The role of the microcirculation is increasingly being recognized in the pathophysiology of cardiovascular disease.[1,2] The microcirculation is a major site of damage in most target organs of cardiovascular disease, such as the heart, brain, and kidney. Both functional and structural alterations in the small arteries, arterioles, and capillaries are the basis of target organ damage. Furthermore, the microcirculation is the major site of control of vascular resistance. This makes it a central player in the etiopathogenesis of diseases characterized by an increased vascular resistance, such as hypertension.

Detailed mechanistic studies in both humans and animal models of cardiovascular disease have revealed the nature of microcirculatory dysfunction. Large-scale epidemiological studies in the last 2 decades have identified the associations among deranged microvascular perfusion, structure, target organ damage, and subsequent cardiovascular disease.[3] Major technological developments now allow study of the microcirculation both in mechanistic and epidemiological studies. The purpose of this Brief Review is to provide a critical appraisal of these developments and their particular impact on hypertension research.

Assessment of the Microcirculation

The Table gives an overview of the major methods to assess the microcirculation. Intravital microscopy has been used by many groups in experimental models to study microcirculatory (dys)function. It has been the primary technology underlying our present knowledge of microcirculatory function in health and disease. Intravital microscopy is the optical imaging of living organisms. The tissue to be studied is prepared by surgical techniques and microscopes, usually in combination with high quality video recorders, is used to visualize the microcirculation. Originally this technique was used in relatively transparent tissues like the bat wing, hamster cheek pouch, or rat mesentery. Later developments using trans- and epi-illumination have allowed wider access to the microcirculation of other tissues, such as skeletal muscle, the brain, and the heart. The recent introduction of molecular imaging probes now allows detailed analyses of molecular mechanisms in microcirculatory control.[4]

The major advantage of intravital microscopy is that it allows direct and precise observation of the microcirculation and its dynamics in vivo. However, the access to tissues usually requires surgery and anesthesia, thus limiting the applicability in human studies. New techniques for video microscopic examinations have been introduced in the past 2 decades that do not require surgery and anesthesia. These techniques are based on the use of orthogonal polarization spectral or sidestream darkfield imaging.[5–7] Both devices use the principle that green light illuminates the depth of a tissue and that the scattered green light is absorbed by hemoglobin of red blood cells contained in superficial vessels.[6] These techniques have been applied in humans for the study of various tissues but mostly the cutaneous and sublingual microcirculation. Video recordings by hand-held cameras now allow microcirculatory observations to be made even in epidemiological studies. Parameters used to assess the microcirculation using orthogonal polarization spectral and sidestream darkfield imaging include total vascular density; arteriolar, venular, and capillary density; microvascular flow; and microvascular diameters. A recent article by De Backer et al[8] gives an excellent review on microcirculatory changes assessed by these novel imaging methods in humans. Broekhuizen et al[9] have used sidestream darkfield imaging recently to study the behavior of the endothelial glycocalyx in humans. Endothelial glycocalyx perturbation contributes to increased vascular permeability and has been shown to be involved in the vascular complications of type 2 diabetes mellitus and perhaps other cardiovascular diseases.[9]

Capillaroscopy

Until recently, nailfold capillaroscopy, using rather bulky microscopes, was the standard technique to study the microcirculation in hypertensive patients. Capillaroscopy consists of the direct in vivo observation of skin capillaries using a microscope with an epi-illumination system.[10] Nailfold capillaries are parallel to the surface of the skin, which facilitates their observation. Fluorescent tracers, such as Na-fluorescein and indocyanine green, have been used to improve the image contrast and to study dynamics of the microcirculation in addition to transcapillary diffusion. Abnormal patterns have been observed in diseases affecting the digital skin microvasculature, such as systemic sclerosis, but also in diseases like diabetes mellitus and hypertension.[10,11] Skin capillary density has been consistently found to be 10% to 20% lower in

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Table. Major Methods to Assess the Microcirculation

Intravital microscopy
Capillaroscopy
Retinal imaging
Isolated small arteries
Contrast angiography
MRI
Positron emission tomography
Laser Doppler flowmetry

patients with untreated hypertension, in comparison with normotensive controls.[12–15] This defect might be an early feature of hypertensive disease, as was reported in borderline hypertensives,[16] and even in normotensive offspring of hypertensive parents.[17] He et al[18] showed recently that modest salt reduction can restore capillary density in patients with mild hypertension. Shore and coworkers[19,20] have, in addition to capillary density, measured capillary pressures in the skin of hypertensive individuals. Capillary pressure was higher in both elderly normotensives and hypertensives than in young normotensives, suggesting a significant effect of aging rather than hypertension, per se, on capillary pressure.

Retinal Imaging

A major advantage in large-scale epidemiological studies on the pathophysiology of hypertension has been the introduction of retinal imaging methods.[3,21,22] Hypertensive retinopathy was first described in the 19th century and has been used since then in the diagnosis of the severity of hypertension-induced target organ damage. However, the classic assessment of retinopathy was descriptive and gave no quantitative or mechanistic data on the microcirculatory dysfunction. In the last 2 decades several groups have advanced the technology of retinal microcirculatory image analysis with the use of a nonmydriatic video camera.[22–24] In particular, the advances introduced by Knudtson et al[25] have allowed retinal microcirculatory analysis to become part of both mechanistic and epidemiological studies. A further major technical advance was the introduction of scanning laser Doppler flowmetry, which allows perfusion imaging analysis.[26] This technique also allows determination of the wall thickness and wall: lumen ratio of individual retinal arterioles.[27] A word of caution should be mentioned, in that retinal microvascular dimensions are not only the result of structure but also of function, because measurements cannot be made under fully relaxed conditions.

The retinal microcirculation undergoes a series of pathophysiological changes during and after the development of hypertension. In the initial, vasoconstrictive stage, there is vasospasm and an increase in retinal arteriolar tone.[22] Persistently elevated blood pressure leads to intimal thickening, hyperplasia of the media wall, and hyaline degeneration in the subsequent sclerotic stage. This stage corresponds with more severe areas of arteriolar narrowing and changes in the arteriolar and venular junctions (arteriovenous nicking of nipping).[22] In an even later stage, there is a disruption of the blood-retina barrier with microaneurysms, hemorrhages, necrosis of the smooth muscle and endothelial cells, and retinal ischemia.

Retinal microcirculatory imaging techniques have substantial reproducibility[28,29] and can be used repeatedly in the same individuals for follow-up. Such longitudinal studies have shown that signs of hypertensive retinopathy can be observed already in relatively young individuals without a history of hypertension.[22] These data suggest that retinal arteriolar narrowing may precede the development of hypertension.[30] Retinal microcirculatory analysis has been used for the risk stratification of hypertensive patients because it shows a strong association with the risk to develop stroke,[31] coronary heart disease,[32,33] and renal complications.[34] An autopsy study of patients with stroke showed a close correlation between retinal and cerebral arteriolar changes.[35] At an even more advanced level, retinal microcirculation imaging allows the analysis of arteriolar and venular branching patterns and retinal vascular fractal dimensions.[36] We have suggested previously that abnormal growth and branching of the vascular tree may represent an early genetic or fetal programming-related characteristic of hypertensive-prone individuals.[37]

Advanced Imaging Technologies

The (video)microscopy techniques discussed above allow both structural and functional studies of the microcirculation. Over the past 2 decades, there has been a growing interest in advanced perfusion imaging technologies, such as laser Doppler flowmetry, positron emission tomography, MRI, and angiography. Laser Doppler flowmetry is based on the backscattering of a beam of laser light. The light undergoes changes in wavelength when it hits moving cells. The magnitude and frequency distribution of these changes in wavelength are related to the number and velocity of red blood cells.[10] Laser Doppler flowmetry assesses blood flow of superficial tissue (ie, skin) over a small volume and is accurate for detecting and quantifying relative changes in skin blood flow in response to a given stimulus.[10] Because of spatial variability, the reproducibility of this technique is relatively poor. The more recently developed 2D laser Doppler perfusion imaging, in which a region of skin is progressively scanned, reduces spatial variability. However, it does not provide an exact linear measure of flow.[10] This makes laser Doppler mostly suited to assess microvascular reactivity instead of absolute measurement of microvasculatory structure or flow.

Positron emission tomography has been used for >35 years as a powerful tool to study cardiac physiology.[38] Apart from studies on metabolism, it allows the assessment of myocardial perfusion in combination with molecular studies. Coronary microvascular function was conventionally assessed by studying flow changes detected by thermodilution or intracoronary Doppler flow wires.[38] The invasive nature of these technologies limits their applicability. Positron emission tomography has become an alternative technique to study microvascular function, although it is still used only in highly specialized centers. For a detailed review on positron emission tomography and coronary microvascular function, readers are referred to 2 recent review articles.[38,39]

MRI has undergone major advances in the past years and is now used to study the structure of arteries in various organs, such as the brain and the heart. However, its resolution is still not high enough to assess the microcirculation, because the smallest sizes of vessels that can be measured are in the range of 200 to 300 μm.[40] The same is true for angiography on the basis of computed tomography or dyes and for ultrasound-based methods for vascular imaging. Again, most advances are being made in the area of myocardial microvascular studies. The recent progress in MRI and angiography has been reviewed recently.[40]

Isolated Small Arteries

All of the above-discussed methods to study the microcirculation share an in vivo approach. Isolated small arteries have been used in the past 25 years successfully to study other aspects of microcirculatory behavior in health and disease. Small arteries have been obtained either from surgical procedures or from specific subcutaneous gluteal biopsies.[41–43] A clear advantage of the in vitro approach has been the possibility for detailed structural analyses of the small arteries both in diseased conditions and during pharmacological treatment of patients. With respect to structural alterations, small arteries remodel in hypertension with 2 types of remodeling. Inward eutrophic remodeling is usually found in primary forms of hypertension in humans and rats, whereas inward hypertrophic remodeling has been described in secondary hypertension and hypertension associated with diabetes mellitus.[41–43] These 2 forms of remodeling reflect divergent ways in which small arteries adapt their structure in the face of mechanical and chemical stresses. The mechanisms of these forms of remodeling are still poorly understood but seem to involve growth of both cellular and matrix components of the vessel wall. Low-grade inflammation of the arterial wall and perhaps perivascular fat also plays a role in arterial remodeling.[44–46] The reader is referred to 2 recent reviews for a more in-depth discussion on mechanism of small artery remodeling, as studied by micromyography.[42,43]

Park and Schiffrin[47] have proposed that small artery remodeling may be an early manifestation of target organ damage in hypertension. Small artery structure has important prognostic significance for later cardiovascular events in both hypertensive and normotensive individuals.[42] Although there are limitations to the in vivo relevance of these isolated artery studies, they provide an excellent approach to the study of molecular and cellular mechanisms of microvascular changes in hypertension and cardiovascular disease.

Microcirculatory Dysfunction: Cause or Consequence of Hypertension?

Microcirculatory dysfunction seems to be both a cause and consequence of elevated blood pressure.[2] Arteriolar and capillary rarefaction and small artery remodeling are early hallmarks of hypertension and have been shown to occur already before or early in the onset of primary hypertension in humans or animal models.[2,47] On the other hand, the microcirculation is a primary target of the organ damage caused by an elevated blood pressure. Microvascular damage is now held responsible for much of the pathology related to cardiac, brain, and renal dysfunction in hypertension.[48] The microcirculation is part of a vicious cycle that initiates, maintains, and amplifies high blood pressure if it is not treated adequately.[49]

The most rigorous way to investigate the behavior of this vicious cycle is to follow the dynamics of the microcirculation throughout life in a population at risk to develop hypertension. Ideally, such a population should be followed up from birth. In animal models like the spontaneously hypertensive rat, such studies have been performed,[50] but the most challenging study is, of course, a human one. A beginning of such studies has been made on the basis of retinal imaging and orthogonal polarization spectral video-capillaroscopy. In 6- to 8-year–old children, those with the higher quartiles of blood pressure had significantly narrower retinal arterioles.[51] Recent studies showed that low birth weight children, who are at risk to develop hypertension later in life, have a narrower retinal arteriolar caliber at the ages of 6 and 12 years.[52,53] Earlier studies had already associated low birth weight with capillary rarefaction in both prepubertal children and adults.[54–57] Surprisingly, low birth weight infants do not have capillary rarefaction at birth.[58] In low birth weight infants, capillary density may be even higher because of the relative systemic hypoxia that these infants experienced in utero.[56] Basal capillary density decreases progressively after the first week of life because of a process of pruning. It may be speculated that low birth weight infants undergo a process of capillary hyperpruning because of a relative hyperoxia of the extrauterine environment, together with supplemental oxygen in the postnatal period of preterm infants. Alternatively, a "catch-up" process with abundant availability of nutrients may cause capillary hyperpruning.[59] Follow-up studies on the neonatal cohort described by D'Souza et al[58] have to be awaited to decide on this hypothesis.

Genetic Determinant of Microcirculatory Phenotypes

The genetic components of hypertensive disease have been the focus of recent intense research efforts. Apart from several rare forms of monogenic causes, hypertension appears to be associated with subtle changes in a range of genes. Recent genome-wide association studies indicate that perhaps $>$30 genes can contribute, each to a small degree, to average blood pressure values in a population.[60,61] Because blood pressure is a highly variable phenotype in an individual, it can be speculated that more robust underlying phenotypes, such as microvascular structure, give better correlations. With respect to the microcirculation, recent studies have focused on the genetic influence on the structure of the retinal microcirculation. There is a strong heritability for the retinal arteriolar and venular caliber.[62] Genome-wide association studies have revealed several loci that were significantly associated with retinal arteriolar and venular caliber.[62–64] However, there was no overlap in the specific loci found in the 3 published genome-wide association studies. This may suggest a lack of power or may indicate regional differences, because the studies were based on populations from different parts of the world. Another approach in genetic studies is the candidate gene approach. Using this approach, we found recently that diameters of the retinal arterioles are associated

with the 1675G/A polymorphism in the angiotensin type 2 receptor gene.[65]

Conclusion

The microcirculation is both a major site of vascular resistance control and of target organ damage in hypertensive disease. Evidence from animal, clinical, and epidemiological studies has confirmed its essential role in the pathogenesis of hypertension. Major advances in technology now allow the noninvasive study of various aspects of the microcirculation in clinical and even population-based research. Such studies have revealed the major phenotypic microcirculatory changes in hypertension, such as arteriolar narrowing, capillary rarefaction, and altered branching patterns. Future research should focus on the mechanisms underlying these changes in microcirculatory phenotype, as well as on how these are influenced by drug treatment.

The combination of methods of phenotyping the microcirculation discussed in this Brief Review now allows this approach. Intravital microscopy, particularly in combination with molecular imaging probes, remains the state-of-the-art approach for fundamental mechanistic studies on microvascular dynamics in health and (cardiovascular) disease. Retinal imaging has opened a window on the microcirculation for clinical and population-based studies, thus allowing large-scale investigations on genetic and environmental influences on the microcirculation. Further technological developments in the area of optical imaging will refine our possibilities to phenotype the microcirculation.

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None.

References

1. Levy BI, Schiffrin EL, Mourad JJ, Agostini D, Vicaut E, Safar ME, Struijker-Boudier HA. Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus. *Circulation*. 2008;118:968–976.
2. Struijker-Boudier HA, Rosei AE, Bruneval P, Camici PG, Christ F, Henrion D, Levy BI, Pries A, Vanoverschelde JL. Evaluation of the microcirculation in hypertension and cardiovascular disease. *Eur Heart J*. 2007;28:2834–2840.
3. Strain WD, Adingupu DD, Shore AC. Microcirculation on a large scale: techniques, tactics and relevance of studying the microcirculation in larger population samples. *Microcirculation*. 2011;19:37–46.
4. Fukumura D, Duda DG, Munn LL, Jain RK. Tumor microvasculature and microenvironment: novel insights through intravital imaging in preclinical models. *Microcirculation*. 2010;17:206–225.
5. Awan ZA, Wester T, Kvernebo K. Human microvascular imaging: a review of skin and tongue videomicroscopy techniques and analysing variables. *Clin Physiol Funct Imaging*. 2009;30:79–88.
6. De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, Ince C. How to evaluate the microcirculation: report of a round table conference. *Crit Care*. 2007;11:R101.
7. Goedhart PT, Khalilzada M, Bezemer R, Merza J, Ince C. Sidestream dark field (SDF) imaging: a novel stroboscopic led ring-based imaging modality for clinical assessment of the microcirculation. *Opt Express*. 2007;15:15101–15114.
8. De Backer D, Donadello K, Taccone FS, Ospina-Tascon G, Salgado D, Vincent JL. Microcirculatory alterations: potential mechanisms and implications for therapy. *Ann Intensive Care*. 2011;1:27.
9. Broekhuizen LN, Lemkes BA, Mooij HL, Meuwese MC, Verberne H, Holleman F, Schlingemann RO, Nieuwdorp M, Stroes ES, Vink H. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. *Diabetologia*. 2010;53:2646–2655.
10. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation*. 2011;19:47–64.
11. De Boer MP, Meijer RI, Wijnstok NJ, Jonk AM, Houben AJ, Stehouwer CD, Smulders YM, Eringa EC, Serne EH. Microvascular dysfunction: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Microcirculation*. 2011;19:5–18.
12. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. *Hypertension*. 1999;33:998–1001.
13. Debbabi H, Uzan L, Mourad JJ, Safar M, Levy BI, Tibirica E. Increased skin capillary density in treated essential hypertensive patients. *Am J Hypertens*. 2006;19:477–483.
14. Prasad A, Dunnill GS, Mortimer PS, MacGregor GA. Capillary rarefaction in the forearm skin in essential hypertension. *J Hypertens*. 1995;13:265–268.
15. Serne EH, Gans RO, ter Maaten JC, Tangelder GJ, Donker AJ, Stehouwer CD. Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. *Hypertension*. 2001;38:238–242.
16. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension*. 1999;34:655–658.
17. Antonios TF, Rattray FM, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Rarefaction of skin capillaries in normotensive offspring of individuals with essential hypertension. *Heart*. 2003;89:175–178.
18. He FJ, Marciniak M, Markandu ND, Antonios TF, MacGregor GA. Effect of modest salt reduction on skin capillary rarefaction in white, black, and asian individuals with mild hypertension. *Hypertension*. 2010;56:253–259.
19. Fegan PG, Tooke JE, Gooding KM, Tullett JM, MacLeod KM, Shore AC. Capillary pressure in subjects with type 2 diabetes and hypertension and the effect of antihypertensive therapy. *Hypertension*. 2003;41:1111–1117.
20. James MA, Tullett J, Hemsley AG, Shore AC. Effects of aging and hypertension on the microcirculation. *Hypertension*. 2006;47:968–974.
21. Grassi G, Schmieder RE. The renaissance of the retinal microvascular network assessment in hypertension: new challenges. *J Hypertens*. 2011;29:1289–1291.
22. Wong TY, Mitchell P. Hypertensive retinopathy. *N Engl J Med*. 2004;351:2310–2317.
23. Harazny JM, Ritt M, Baleanu D, Ott C, Heckmann J, Schlaich MP, Michelson G, Schmieder RE. Increased wall: lumen ratio of retinal arterioles in male patients with a history of a cerebrovascular event. *Hypertension*. 2007;50:623–629.
24. Hughes AD, Martinez-Perez E, Jabbar AS, Hassan A, Witt NW, Mistry PD, Chapman N, Stanton AV, Beevers G, Pedrinelli R, Parker KH, Thom SA. Quantification of topological changes in retinal vascular architecture in essential and malignant hypertension. *J Hypertens*. 2006;24:889–894.
25. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res*. 2003;27:143–149.
26. Ritt M, Harazny JM, Ott C, Schlaich MP, Schneider MP, Michelson G, Schmieder RE. Analysis of retinal arteriolar structure in never-treated patients with essential hypertension. *J Hypertens*. 2008;26:1427–1434.
27. Harazny JM, Raff U, Welzenbach J, Ott C, Ritt M, Lehmann M, Michelson G, Schmieder RE. New software analyses increase the reliability of measurements of retinal arterioles morphology by scanning laser Doppler flowmetry in humans. *J Hypertens*. 2011;29:777–782.
28. Couper DJ, Klein R, Hubbard LD, Wong TY, Sorlie PD, Cooper LS, Brothers RJ, Nieto FJ. Reliability of retinal photography in the assessment of retinal microvascular characteristics: the Atherosclerosis Risk in Communities Study. *Am J Ophthalmol*. 2002;133:78–88.
29. Liu Y-P, Richart T, Jin Y, Struijker-Boudier HAJ, Staessen JA. Retinal arteriolar and venular phenotypes in a Flemish population: reproducibility and correlates. *Artery Research*. 2011;5:72–79.

30. Ikram MK, Witteman JC, Vingerling JR, Breteler MM, Hofman A, de Jong PT. Retinal vessel diameters and risk of hypertension: the Rotterdam Study. *Hypertension*. 2006;47:189–194.
31. Witt N, Wong TY, Hughes AD, Chaturvedi N, Klein BE, Evans R, McNamara M, Thom SA, Klein R. Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. *Hypertension*. 2006;47:975–981.
32. Duncan BB, Wong TY, Tyroler HA, Davis CE, Fuchs FD. Hypertensive retinopathy and incident coronary heart disease in high risk men. *Br J Ophthalmol*. 2002;86:1002–1006.
33. Sairenchi T, Iso H, Yamagishi K, Irie F, Okubo Y, Gunji J, Muto T, Ota H. Mild retinopathy is a risk factor for cardiovascular mortality in japanese with and without hypertension: the Ibaraki Prefectural Health Study. *Circulation*. 2011;124:2502–2511.
34. Sabanayagam C, Tai ES, Shankar A, Lee J, Sun C, Wong TY. Retinal arteriolar narrowing increases the likelihood of chronic kidney disease in hypertension. *J Hypertens*. 2009;27:2209–2217.
35. Goto I, Katsuki S, Ikui H, Kimoto K, Mimatsu T. Pathological studies on the intracerebral and retinal arteries in cerebrovascular and noncerebrovascular diseases. *Stroke*. 1975;6:263–269.
36. Cheung CY, Tay WT, Mitchell P, Wang JJ, Hsu W, Lee ML, Lau QP, Zhu AL, Klein R, Saw SM, Wong TY. Quantitative and qualitative retinal microvascular characteristics and blood pressure. *J Hypertens*. 2011;29:1380–1391.
37. le Noble FA, Stassen FR, Hacking WJ, Struijker Boudier HA. Angiogenesis and hypertension. *J Hypertens*. 1998;16:1563–1572.
38. Al-Mallah MH, Sitek A, Moore SC, Di Carli M, Dorbala S. Assessment of myocardial perfusion and function with pet and pet/ct. *J Nucl Cardiol*. 2010;17:498–513.
39. Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med*. 2007;356:830–840.
40. Porto I, Hamilton-Craig C, Brancati M, Burzotta F, Galiuto L, Crea F. Angiographic assessment of microvascular perfusion: myocardial blush in clinical practice. *Am Heart J*. 2010;160:1015–1022.
41. Aalkjaer C, Heagerty AM, Petersen KK, Swales JD, Mulvany MJ. Evidence for increased media thickness, increased neuronal amine uptake, and depressed excitation: contraction coupling in isolated resistance vessels from essential hypertensives. *Circ Res*. 1987;61:181–186.
42. Rizzoni D, Muiesan ML, Porteri E, De Ciuceis C, Boari GE, Salvetti M, Paini A, Rosei EA. Vascular remodeling, macro- and microvessels: therapeutic implications. *Blood Press*. 2009;18:242–246.
43. Schiffrin EL. Vascular remodeling in hypertension: mechanisms and treatment. *Hypertension*. 2012;59:367–374.
44. Aghamohammadzadeh R, Withers S, Lynch F, Greenstein A, Malik R, Heagerty A. Perivascular adipose tissue from human systemic and coronary vessels: the emergence of a new pharmacotherapeutic target. *Br J Pharmacol*. 2012;165:670–682.
45. Matsuda M, Shimomura I, Sata M, Arita Y, Nishida M, Maeda N, Kumada M, Okamoto Y, Nagaretani H, Nishizawa H, Kishida K, Komuro R, Ouchi N, Kihara S, Nagai R, Funahashi T, Matsuzawa Y. Role of adiponectin in preventing vascular stenosis: the missing link of adipovascular axis. *J Biol Chem*. 2002;277:37487–37491.
46. Takaoka M, Suzuki H, Shioda S, Sekikawa K, Saito Y, Nagai R, Sata M. Endovascular injury induces rapid phenotypic changes in perivascular adipose tissue. *Arterioscler Thromb Vasc Biol*. 2010;30:1576–1582.
47. Park JB, Schiffrin EL. Small artery remodeling is the most prevalent (earliest?) form of target organ damage in mild essential hypertension. *J Hypertens*. 2001;19:921–930.
48. Safar ME, Struijker-Boudier HA. Cross-talk between macro- and microcirculation. *Acta Physiol (Oxf)*. 2010;198:417–430.
49. Cohuet G, Struijker-Boudier H. Mechanisms of target organ damage caused by hypertension: therapeutic potential. *Pharmacol Ther*. 2006;111:81–98.
50. Struijker Boudier HA, le Noble JL, Messing MW, Huijberts MS, le Noble FA, van Essen H. The microcirculation and hypertension. *J Hypertens Suppl*. 1992;10(suppl)S147–S156.
51. Mitchell P, Cheung N, de Haseth K, Taylor B, Rochtchina E, Islam FM, Wang JJ, Saw SM, Wong TY. Blood pressure and retinal arteriolar narrowing in children. *Hypertension*. 2007;49:1156–1162.
52. Gopinath B, Baur LA, Wang JJ, Teber E, Liew G, Cheung N, Wong TY, Mitchell P. Smaller birth size is associated with narrower retinal arterioles in early adolescence. *Microcirculation*. 2010;17:660–668.
53. Mitchell P, Liew G, Rochtchina E, Wang JJ, Robaei D, Cheung N, Wong TY. Evidence of arteriolar narrowing in low-birth-weight children. *Circulation*. 2008;118:518–524.
54. Chapman N, Mohamudally A, Cerutti A, Stanton A, Sayer AA, Cooper C, Barker D, Rauf A, Evans J, Wormald R, Sever P, Hughes A, Thom S. Retinal vascular network architecture in low-birth-weight men. *J Hypertens*. 1997;15:1449–1453.
55. Ijzerman RG, van Weissenbruch MM, Voordouw JJ, Yudkin JS, Serne EH, Delemarre-van de Waal HA, Stehouwer CD. The association between birth weight and capillary recruitment is independent of blood pressure and insulin sensitivity: a study in prepubertal children. *J Hypertens*. 2002;20:1957–1963.
56. Sasongko MB, Wong TY, Wang JJ. Retinal arteriolar changes: Intermediate pathways linking early life exposures to cardiovascular disease? *Microcirculation*. 2010;17:21–31.
57. Serne EH, Stehouwer CD, ter Maaten JC, ter Wee PM, Donker AJ, Gans RO. Birth weight relates to blood pressure and microvascular function in normal subjects. *J Hypertens*. 2000;18:1421–1427.
58. D'Souza R, Raghuraman RP, Nathan P, Manyonda IT, Antonios TF. Low birth weight infants do not have capillary rarefaction at birth: implications for early life influence on microcirculation. *Hypertension*. 2011;58:847–851.
59. Hellstrom A, Ley D, Hansen-Pupp I, Niklasson A, Smith L, Lofqvist C, Hard AL. New insights into the development of retinopathy of prematurity: importance of early weight gain. *Acta Paediatr*. 2010;99:502–508.
60. The International Consortium for Blood Pressure Genome Wide Association Studies. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.
61. Salvi E, Kutalik Z, Glorioso N, Benaglio P, Frau F, Kuznetsova T, Arima H, Hoggart C, Tichet J, Nikitin YP, Conti C, Seidlerova J, Tikhonoff V, Stolarz-Skrzypek K, Johnson T, Devos N, Zagato L, Guarrera S, Zaninello R, Calabria A, Stancanelli B, Troffa C, Thijs L, Rizzi F, Simonova G, Lupoli S, Argiolas G, Braga D, D'Alessio MC, Ortu MF, Ricceri F, Mercurio M, Descombes P, Marconi M, Chalmers J, Harrap S, Filipovsky J, Bochud M, Iacoviello L, Ellis J, Stanton AV, Laan M, Padmanabhan S, Dominiczak AF, Samani NJ, Melander O, Jeunemaitre X, Manunta P, Shabo A, Vineis P, Cappuccio FP, Caulfield MJ, Matullo G, Rivolta C, Munroe PB, Barlassina C, Staessen JA, Beckmann JS, Cusi D. Genomewide association study using a high-density single nucleotide polymorphism array and case-control design identifies a novel essential hypertension susceptibility locus in the promoter region of endothelial no synthase. *Hypertension*. 2012;59:248–255.
62. Sun C, Zhu G, Wong TY, Hewitt AW, Ruddle JB, Hodgson L, Montgomery GW, Young TL, Hammond CJ, Craig JE, Martin NG, He M, Mackey DA. Quantitative genetic analysis of the retinal vascular caliber: the Australian Twins Eye Study. *Hypertension*. 2009;54:788–795.
63. Ikram MK, Sim X, Jensen RA, Cotch MF, Hewitt AW, Ikram MA, Wang JJ, Klein R, Klein BE, Breteler MM, Cheung N, Liew G, Mitchell P, Uitterlinden AG, Rivadeneira F, Hofman A, de Jong PT, van Duijn CM, Kao L, Cheng CY, Smith AV, Glazer NL, Lumley T, McKnight B, Psaty BM, Jonasson F, Eiriksdottir G, Aspelund T, Harris TB, Launer LJ, Taylor KD, Li X, Iyengar SK, Xi Q, Sivakumaran TA, Mackey DA, Macgregor S, Martin NG, Young TL, Bis JC, Wiggins KL, Heckbert SR, Hammond CJ, Andrew T, Fahy S, Attia J, Holliday EG, Scott RJ, Islam FM, Rotter JI, McAuley AK, Boerwinkle E, Tai ES, Gudnason V, Siscovick DS, Vingerling JR, Wong TY. Four novel loci (19q13, 6q24, 12q24, and 5q14) influence the microcirculation in vivo. *PLoS Genet*. 2010;6:e1001184.
64. Xing C, Klein BE, Klein R, Jun G, Lee KE, Iyengar SK. Genome-wide linkage study of retinal vessel diameters in the Beaver Dam eye study. *Hypertension*. 2006;47:797–802.
65. Liu YP, Kuznetsova T, Thijs L, Jin Y, Schmitz B, Brand SM, Brand E, Manunta P, Bianchi G, Struijker-Boudier H, Staessen JA. Are retinal microvascular phenotypes associated with the 1675g/a polymorphism in the angiotensin II type-2 receptor gene? *Am J Hypertens*. 2011;24:1300–1305.