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Assessing Microvascular Function in Humans from a Chronic Disease Perspective

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ABSTRACT

Microvascular dysfunction (MVD) is considered a crucial pathway in the development and progression of cardiometabolic and renal disease and is associated with increased cardiovascular mortality. MVD often coexists with or even precedes macrovascular disease, possibly due to shared mechanisms of vascular damage, such as inflammatory processes and oxidative stress. One of the first events in MVD is endothelial dysfunction. With the use of different physiologic or pharmacologic stimuli, endothelium-dependent (micro)vascular reactivity can be studied. This reactivity depends on the balance between various mediators, including nitric oxide, endothelin, and prostanoids, among others. The measurement of microvascular (endothelial) function is important to understand the pathophysiologic mechanisms that contribute to MVD and the role of MVD in the development and progression of cardiometabolic/renal disease. Here, we review a selection of direct, noninvasive techniques for measuring human microcirculation, with a focus on methods, interpretation, and limitations from the perspective of chronic cardiometabolic and renal disease.

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Noninvasive assessment of large arterial structure and function has been revolutionized by the development of vascular ultrasound. This has enabled broad application of measurement of carotid artery intima-media thickness and brachial artery flow-mediated, endothelium-dependent vasodilation in observational studies and clinical trials. In contrast, broadly applicable assessment of microvascular structure and function has lagged behind, because such measurements are technically demanding. Thus, assessment of microvascular function has relied, to an important extent, on the use of indirect biomarkers of microvascular endothelial function such as albuminuria and plasma or serum levels of molecules produced by the endothelium (*e.g.*, vWf and soluble adhesion molecules). The interpretation, merits, and limitations of these biomarkers have been reviewed elsewhere.[1,2]

Technologic advances have now made noninvasive, direct assessment of microvascular function possible. This is important, because microvascular dysfunction (MVD) is considered a crucial pathway in the development and progression of both cardiometabolic[3–5] and renal disease,[6] and is associated with increased (cardiovascular) mortality.[7,8] MVD often coexists with or even precedes macrovascular disease, possibly due to shared mechanisms of vascular damage.[9] A key player in MVD is the endothelium.[10,11] Classically, (micro)vascular endothelial function relates to endothelium-dependent vasodilation in response to physiologic or pharmacologic stimuli, which depends on the balance between various mediators such as nitric oxide (NO), endothelin, prostanoids, *etc.*[12] Nevertheless, microvascular endothelium regulates not only vasomotor tone, but also permeability, coagulation, fibrinolysis, and proliferation.

Here, we review a selection of direct, noninvasive measurements of the microcirculation, with a focus on methods, interpretation, and limitations from the perspective of chronic cardiometabolic and renal disease.

THE MICROCIRCULATION: STRUCTURE AND FUNCTION

The microcirculation can be anatomically defined as blood vessels with a diameter$<$200–150 μm and comprises arterioles, capillaries, and venules. The function of the microcirculation is to distribute nutrients within, and collect waste products from, tissues. In addition, the microcirculation is involved in BP regulation because it is the major site

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of control of vascular resistance.[13] Arterioles distribute blood within tissues according to local metabolic demand, using vasomotion as an essential mechanism. The actual exchange of fluid and solutes such as nutrients and hormones with the interstitium takes place in capillaries. Small venules not only collect capillary blood, but also play a role in determining capillary pressure. In addition, in many tissues, (postcapillary) venules are the preferential site for adhesion and diapedesis of leukocytes from blood into tissue.[14,15]

Here, we define microvascular function as any activity of microvessels either in the basal state or after stimulation. Microvascular function is the result of vessel wall components' (smooth muscle cells, matrix, endothelium) structure and function, which are inextricably linked to neurogenic and local metabolic influences. Nevertheless, microvascular reactivity to various stimuli is often referred to as a "marker" of endothelial function, because it importantly involves endothelial vasomotor factors.

EXPLORING THE MICROCIRCULATION IN HUMANS

Noninvasive assessment of microvascular function is limited to a few organs: skin (using videomicroscopy, laser-Doppler flowmetry/imaging, or transcutaneous oxygen measurements), bulbar conjunctiva (using videomicroscopy), sublingual mucosa (using videomicroscopy), and retina (using fundus photography/videomicroscopy). Generalization of findings from one tissue to another should of course be done with caution. Although general functions of arterioles, capillaries, and venules are the same throughout the body, the organization of the microcirculation and the control of blood flow differ among tissues, depending on metabolic demand and specific organ functions. In addition, the position of a vessel segment in the vascular tree determines endothelial cell phenotype.[15–17] Factors such as flow type, shear stress, local metabolic demands, and epigenetics shape the phenotype of the endothelium in the different parts of the (micro)circulation. For example, saphenous veins used in coronary artery bypass grafting and thus exposed to arterial flow conditions have been shown to increase endothelial NO synthase and reduce thrombomodulin production.[17] Also, the lack of correlation between endothelial function measured in conduit arteries (using flow-mediated dilation) and in the microcirculation (*e.g.*, retinal arteriolar dilation, postocclusive hyperemia in skin, and retinal arteriolar/venular diameters)[18–20] may be related to differences in endothelial phenotype in the different parts of the vascular tree.

Capillary Microscopy

Skin is a unique site for simple and reproducible assessment of capillary structure and function, where intravital capillaroscopy can be used to directly visualize perfused nutritive capillaries. At the finger and toe nailfold, capillaries run in parallel to the skin surface, which enables evaluation of capillary morphology and measurement of blood flow and pressure. In all other parts of the skin, capillaries are orientated perpendicularly to skin surface, enabling quantification of capillary density. Only erythrocyte-filled capillaries can be visualized without dyes, using a bench-top or handheld digital videomicroscope with blue or green illumination (to enhance contrast of red blood cells) and a system magnification of approximately 100×. Classically, capillaries are visualized in the skin of the dorsal phalanges of the third or fourth finger, approximately 5 mm proximal to the nailfold. Besides baseline capillary density, functional capillary recruitment (increase in capillary density after arterial occlusion) and the maximum capillary density (during venous occlusion) can be assessed off-line manually or semiautomatically[21,22] (Figure 1).

Functional capillary recruitment results from upstream arteriolar dilation involving a myogenic and endothelial response, and local metabolic factors. Maximal capillary recruitment during venous occlusion results from passive trapping of erythrocytes in the capillaries. Both recruitment capacities are physiologically relevant, because they correlate inversely with insulin resistance and BP.[23–25] In addition, several studies have shown that microvascular responses observed in skin parallel those in muscle. For example, insulin augments capillary recruitment in both skin and muscle,[26,27] whereas the presence of obesity or increased free fatty acid levels attenuates capillary recruitment.[28,29] Capillary density changes may occur early and precede the occurrence of disease. For example, capillary densities and recruitment were lower in normotensive individuals with a family history of hypertension and in borderline hypertensive individuals versus controls.[30,31] In more advanced disease, such as type 2 diabetes, hypertension, and advanced CKD, capillary rarefaction is also seen.[21,32,33] In a healthy cohort (mean age approximately 62±5 years) it was shown that a diet with high intake of sweets was associated with lower capillary densities as compared with a diet with high intake of oil, poultry, and fish.[34] In addition, in a population-based study (mean age approximately 60±9 years) we found that lower skin capillary density was independently associated with the presence of albuminuria, supporting a role of capillary rarefaction in the pathogenesis of albuminuria.[35] In summary, these data suggest that skin capillary density and, in particular, recruitment capacity are associated with relevant physiologic outcomes. Changes can be measured in an early phase, before disease is clinically apparent. Reduced capillary recruitment often parallels other measures of MVD, *e.g.*, in skin[36] or in the kidney (albuminuria).

Laser-Doppler Flowmetry

Moving red blood cells in the superficial skin microvasculature give rise to a Doppler shift of monochromatic laser light, which is proportional to the concentration and speed of the blood cells. With use of this principle, relative changes in skin metabolic and thermoregulatory blood flow can be measured in a single spot (approximately 1 mm^3 of skin) or in a larger skin area with

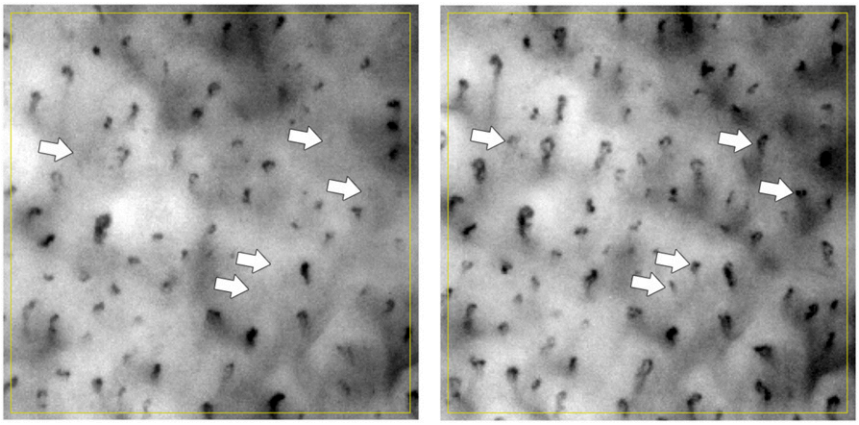

Figure 1. Capillary density in skin on the dorsum of the finger. Images are stills from videomicroscopy clips of exactly the same visual field (1 mm^2 of skin). Left: Baseline capillary density. Right: Capillary recruitment during postocclusive reactive hyperemia. The white arrows represent examples of nonperfused capillaries under baseline conditions that are recruited during postocclusive reactive hyperemia.

laser-Doppler perfusion imaging, revealing spatial heterogeneity in microvascular perfusion.[37] The laser-Doppler signal comes predominantly from small arterioles and venules, and to a lesser extent from capillaries.[38]

Baseline skin blood flow registrations can be used to evaluate flowmotion. Flowmotion is the result of vasomotion, an important component of microvascular function characterized by rhythmic changes in (precapillary) arteriolar diameter. Vasomotion leads to optimal flow distribution to various tissue regions for delivery of oxygen and nutrients,[39,40] and reduces hydraulic resistance.[41] The rhythmic changes in the perfusion signal can be analyzed with time-frequency methods (*e.g.*, Fourier or Wavelet) to distinguish the contribution of different frequency domains to the signal. Typically, five domains can be distinguished, which relate to cardiac and breathing activity, and to (local) endothelial, myogenic, and neurogenic activity.[42] Interest in flowmotion research in the clinical setting is relatively new. Small mechanistic studies have shown that flowmotion can be enhanced by insulin or after a meal,[43,44] and that these reactions are diminished in obesity. In untreated hypertensive subjects, flowmotion is augmented and normalizes after treatment of hypertension.[45] In several other diseases, *e.g.*, peripheral arterial occlusive disease, diabetes, CKD with or without dialysis, or hypercholesterolemia, flowmotion has been found to be attenuated.[46] In a population-based study we have shown that age and waist circumference are inversely, and BP is positively, associated with flowmotion, independent of various confounders.[47] Vaso/flowmotion is undoubtedly an important function of the microcirculation. However, more study is needed to understand how cardiometabolic risk factors affect flowmotion signals. In addition, methodologic standardization is required for the calculation of the spectral value of the different frequency intervals.

Stimulated skin blood flow can also be measured, and gives reproducible measures of microvascular (maximal) response capacity.[48] Both postocclusive and heat-induced reactive hyperemia are partly endothelium dependent[49,50] (Figure 2). Next, endothelium-dependent and -independent reactivity can be measured as responses to acetylcholine (Ach) or sodium nitroprusside, respectively, applied with iontophoresis or microdialysis.[51,52] Stimulated skin blood flow responses have been studied extensively. In healthy volunteers, the Ach- or heat-induced vasodilator response correlated with insulin sensitivity, but not with BP.[23,25] In cross-sectional studies, the Ach-response has been found to be reduced in adults with obesity,[53] but not in obese adolescents or overweight adults.[54,55] Hypertensive, as compared with normotensive, individuals also show a reduced Ach-response.[56,57] Several studies on both type 1 and 2 diabetes have shown reduced Ach- and heat-induced vasodilation, which is worse when complications are present.[58–61] These vasodilator responses are inversely related to the level of glycemic control, and improve with intensified glucose control.[58,62] In a population-based study, we have recently shown that the heat-induced vasodilator response is attenuated in prediabetes and even more in subjects with type 2 diabetes. This vasodilator response was inversely associated with fasting glucose levels, 2 hours postglucose load levels, and hemoglobin A1c levels, also after extensive adjustment for potential confounders.[63] Finally, in patients with more advanced stages of disease, *e.g.*, peripheral arterial occlusive disease, ESRD, or coronary artery disease, these skin vasodilator responses are reduced, but can be improved after treatment.[64–66]

In small studies, the skin vasodilator responses to arterial occlusion,[67] heating, and Ach[68] have been shown to be reduced in diabetic individuals and hypertensive patients with albuminuria, although contradicting results in relation to Ach exist.[69] Similarly, reduced skin vasodilator responses have been observed in individuals with advanced CKD.[70] However, for earlier CKD stages, results are unclear.[71]

In conclusion, skin (endothelium-dependent) vasodilator responses are easy-to-use, sensitive, and physiologically relevant measures of microvascular function. They can be used to detect early changes, even before disease is clinically apparent.

Retinal Imaging

Retinal imaging allows investigation of *in vivo* structure and function of arterioles, venules, and capillaries. Since the early 1920s, fundus photography has played a prominent role in diagnosis and follow-up of eye diseases. The widespread availability of this technique has facilitated its use in many mechanistic and

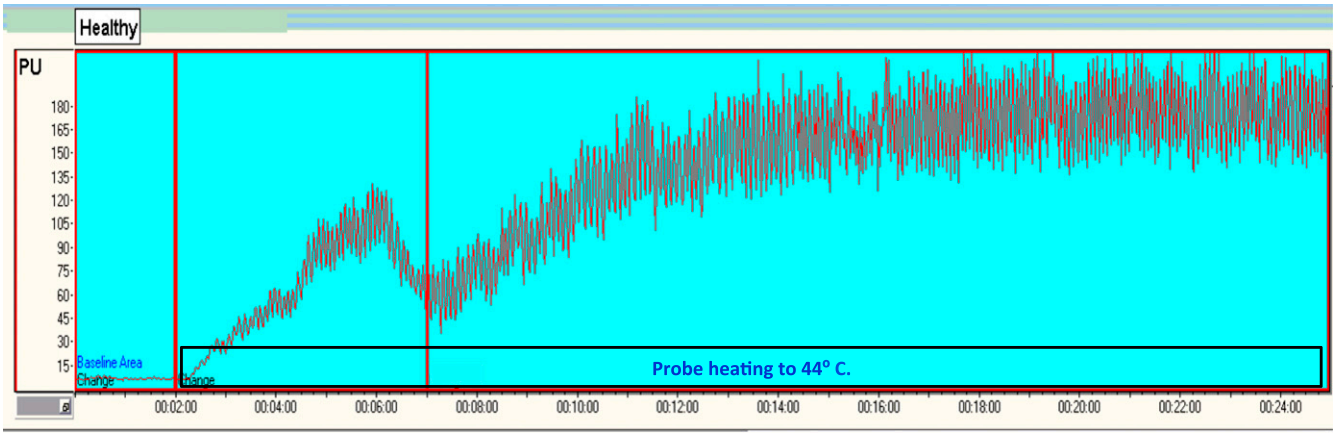

Figure 2. Typical registration of skin microvascular perfusion, measured with laser-Doppler flowmetry, before and during local heating of the skin in a healthy volunteer. After 2 minutes of baseline flow registration, skin heating to 44°C is started for 23 minutes. Time (minute) is depicted on the *x* axis and skin perfusion (arbitrary perfusion units, PU) on the *y* axis. The heat-induced skin hyperemic response is expressed as the percentage increase in average perfusion units during the 23-minute heating phase over the average baseline perfusion units.

epidemiologic studies. Widely used microvascular variables are the central retinal arteriolar/venular equivalents, presented separately or as a ratio (arteriolar venular ratio). Besides diameters, other measures of the retinal microvascular network have been studied, *e.g.*, tortuosity, bifurcation angles and optimality, and fractal dimensions.[72,73] Mechanisms of changes in retinal vessel diameters can be both functional and structural.[74,75] For arterioles this involves changes in endothelial vasodilators (*e.g.*, NO)[76] and constrictors, and BP-related remodeling of the vessel wall.[74,75] For venular widening, inflammatory signals and endothelial dysfunction have been suggested to be involved.[74] A limitation of retinal microvascular analyses from a static image may be that vessel diameters change rhythmically due to vasomotion, which increases intra- and interindividual variability of single image diameter assessments. Recent developments in dynamic retinal imaging techniques have introduced the possibility to measure perfusion and microvessel constrictor responses to oxygen breathing or (endothelium-dependent) dilator responses to flicker light[63,76–78] (Figure 3).

There is a very large body of retinal microvascular studies in relation to cardiometabolic/renal risk factors and diseases. These include mechanistic, cross-sectional, population-based cohort, and longitudinal studies. For example, it has consistently been shown, across age groups, that both current and past higher

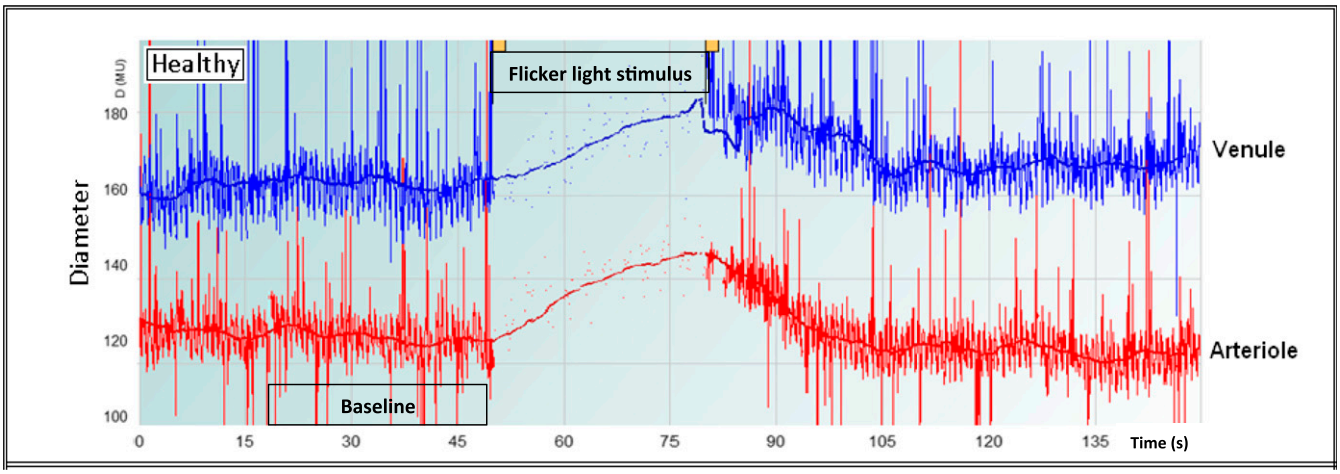

Figure 3. Typical registration of diameter changes of a single retinal arteriole and venule before, during, and after a 30-second flicker light period (*t*=50 to *t*=80 seconds) in a healthy volunteer. Time (seconds) is depicted on the *x* axis and diameter (micrometers) on the *y* axis. The flicker light–induced vasodilator response is expressed as the average increase in diameter during flicker light as a percentage over baseline diameter.

BP are associated with reduced arteriolar diameters.[79–81] Smaller arterioles may not only be an adaptive response to higher BP, but also predict (and possibly contribute to) the development of hypertension.[82] The BP-related reduction in arteriolar diameter seems to be reversible. In hypertensive individuals, frequent fish consumption was associated with wider arteriolar and narrower venular diameters.[83] In addition, 6–12 months of BP treatment resulted in wider arteriolar diameters.[84,85] Finally, lifestyle interventions may also be a treatment option to normalize microvascular diameters.[86]

Data on retinal microvascular diameters and renal function or disease are less consistent. Cross-sectional data from an Asian population-based cohort showed an association between smaller arteriolar diameters and CKD (defined as eGFR of $<$60 ml/min per 1.73 m^2 or the presence of micro/macroalbuminuria) independent of the presence of diabetes and hypertension.[87] In addition, patients with CKD (stage 2–4) with small retinal arteriolar diameters were shown to develop more renal end points (function loss or start of dialysis) as compared with patients with larger arteriolar diameters.[88] However, longitudinal population-based cohort studies did not find associations between baseline retinal arteriolar/venular diameters and incident CKD.[6,89]

Taken together, retinal microvascular diameters are relevant, sensitive, valid, reproducible, and consistent markers of microvascular function.

Flicker light can be used to enhance retinal metabolic activity, which, *via* neurovascular coupling, leads to endothelium-dependent vasodilation (involving NO) and increased blood flow.[76,90] Recently, we reported, in a population-based setting, that the retinal arteriolar dilator response to flicker light was reduced in individuals with prediabetes and type 2 diabetes versus normoglycemic individuals.[63] In small cross-sectional studies, similar findings of reduced retinal endothelial function have been found in individuals with hypertension, obesity, and coronary artery disease.[91–93] Recently, it was shown, in patients with diabetes and/or cardiovascular disease, that retinal endothelial function correlates with creatinine clearance and eGFR.[94] Because follow-up studies on retinal vasoreactivity are scarce, the prognostic value of these measurements remains to be explored. Nevertheless, microvascular (endothelial) reactivity data add valuable (patho)physiologic information to static retinal diameter/morphometry data.

DETERMINANTS OF MVD

As stated above, dysfunction of the microcirculation may occur early and contribute to the development of disease. Several determinants of MVD have been identified (Figure 4).

Genetics

Normotensive offspring of hypertensive parents have structural and/or functional microvascular changes,[95] with a lower number of skin capillaries (rarefaction) and reduced capillary recruitment capacity as compared with matched control individuals.[30] In another study,[96] both skin capillary density and heat-induced hyperemia were reduced in hypertensive individuals with hypertensive versus normotensive parents. Similarly, normotensive offspring of hypertensive parents had lower glomerular filtration

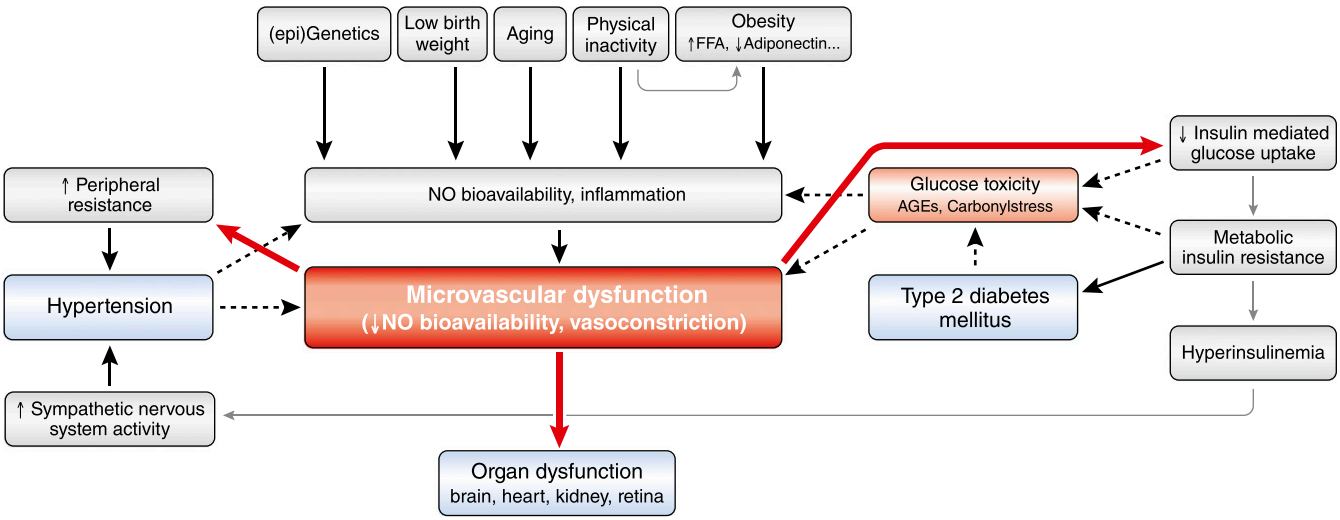

Figure 4. Hypothesis describing determinants contributing to MVD and subsequent organ dysfunction. We here define microvascular (dys)function being the result of vessel wall components' (smooth muscle cells, matrix, endothelium) structure and function, and neurogenic and local metabolic influences. Early MVD leads to impaired insulin-mediated glucose uptake and raised peripheral resistance, which contributes to the development of insulin resistance/type 2 diabetes, and hypertension, respectively. The hyperglycemic milieu and hypertension in turn further aggravate MVD leading to a vicious cycle (dashed arrows). ↑, stimulated (levels of); ↓, reduced (levels of); AGEs, advanced glycation end products; FFA, free fatty acids.

reserve, which seemed to be related to lower NO formation.[97] Importantly, lower glomerular filtration reserve is indicative of renal MVD with glomerular hyperfiltration. The latter may lead to glomerular capillary rarefaction and eventually the development of albuminuria as well as a decline in kidney function. Because hypertension is, at least in part, an inherited condition, the above findings thus suggest that genetic factors may contribute to MVD and that MVD in individuals with hypertension is of primary origin.

Low Birth Weight

Suboptimal intrauterine circumstances may result in low birth weight, which has been linked to cardiometabolic disease in adult life.[98] Endothelial dysfunction, particularly reduced NO synthesis and NO scavenging by reactive oxygen species, may be a mechanism explaining these associations. Indeed, skin endothelium-dependent vasodilation to Ach has been found to be inversely associated with body weight and size in newborns.[99,100] In contrast, functional and structural skin capillary densities seem to be higher in low birth weight as compared with normal birth weight newborns,[101] although at prepubertal age this seems to be reversed.[102] In addition, adults who were born preterm show reduced skin capillary densities,[103] and retinal arteriolar diameters and vascularization.[104,105]

Similarly, low birth weight has been linked to the development of CKD later in life. This risk has been ascribed to a lower nephron number, which may result in an increased susceptibility to glomerular hypertension and a lower glomerular filtration reserve. Indeed, birth weight is positively associated with nephron number in neonates as well as adults.[106]

Physical Inactivity

Two meta-analyses have shown that endothelium-(in)dependent microvascular function is enhanced in both athletes and trained adults versus healthy controls.[107,108] Vice versa, physical inactivity has been found to induce MVD acutely in healthy volunteers after bed rest.[109,110] In addition, population-based cohort studies have shown that less physical activity/increased television viewing time is associated with wider retinal venular diameters.[111,112] These data support the concept that regular exercise is associated with generalized improvement of microvascular function in the absence of disease. Although the exact mechanisms involved remain to be elucidated, increased shear stress/pressure and reduced oxidative stress levels due to physical activity have been proposed to contribute to augmented NO bioavailability and reduced activity of vasoconstrictor pathways.[113,114] In line with these mechanisms of improved endothelial function, the Nurses' Health Study found that higher levels of physical activity were associated with lower albumin-to-creatinine ratio.[115] In addition, in patients with type 1 diabetes, higher levels of leisure-time physical activity were associated with less progression to renal failure (on the basis of urinary albumin excretion rate) and less incidence of microalbuminuria over 6 years of follow up.[116]

Obesity

Many studies have shown that MVD is present in obesity.[117] Already at a young age, obesity is independently associated with smaller retinal arteriolar and wider venular diameters,[118] which continues in adults.[119] Wider retinal venular, but not smaller arteriolar, diameters may predict development of obesity.[120] In addition, skin capillary recruitment capacity[53] and impaired endothelium-dependent microvascular dilation in skin and muscle have been found in obese individuals.[43,121,122] Several mechanisms may be involved in obesity-related MVD. Elevated free fatty acid levels augment skin MVD,[28] and expanded/dysfunctional adipose tissue (*1*) releases inflammatory signals leading to reduced NO and increased endothelin-1 production; and (*2*) leads to changes in adipokine profile (less adiponectin and more leptin, resistin, and angiotensinogen).[117,123] Visceral adipose tissue seems to be the most important source of this endocrine signaling to the microcirculation, but paracrine signaling from perivascular adipose tissue affects microvascular function as well.[124]

Relevant clinical consequences of obesity-related MVD are insulin resistance and raised BP.[117] Subsequently, chronic hyperglycemia contributes to further deterioration of microvascular endothelial function.[125] Raised BP contributes to endothelial dysfunction, arteriolar wall remodeling, and capillary/arteriolar rarefaction.[95] Together, these conditions progressively aggravate each other in a vicious cycle. At the level of the kidney, MVD may lead to increased GFR and renal blood flow with glomerular hyperfiltration.[126] The latter likely contributes to the development of secondary FSGS and loss of kidney function in individuals with (severe) obesity.[127]

Aging

The hallmark of aging is a gradual loss of functional reserve in all organs and tissues, including the (micro)vasculature. Investigating the independent effects of aging on the microcirculation is complex due to interrelationships of aging with increasing levels of cardiometabolic risk factors and incident cardiovascular disease. Longitudinal data from a population-based study showed reduced retinal arteriolar/venular diameters with increasing age, and a history of cardiovascular disease and CKD was associated with a change in venular diameter over time.[128] In another cohort, age was independently associated with skin microvascular flowmotion.[47] These findings in the systemic microcirculation parallel the significant loss of nephrons with aging observed in healthy kidney donors.[129] Oxidative stress and inflammatory processes in the endothelium have been proposed to be the main drivers of MVD in aging.[130]

FUTURE DIRECTIONS

In this brief review, we focused on a few techniques only that are easy to apply, even in large-scale studies (Table 1). New developments may add valuable information to the status of microvascular function. First, the integrity of the

Table 1. Characteristics of a selection of noninvasive measurements of the human microcirculation, including endothelium-dependent (re)activity, which are clinically easy to perform and can be applied to large-scale studies

Technique	Measured Variable	Unit	Duration	Advantages	Disadvantages
Skin videomicroscopy	Capillary density	number/mm^2; %-change	Perf.: baseline, art and ven occlusion: approximately 11 min. Anal.: 15 min per ROI[a]/finger (semiautomatic)[22]; manually: 30 min.	Direct visualization of capillaries; measures functional reactivity	Difficult in dark skin; laborious analyses
Skin laser-doppler flowmetry Flowmotion Heat-induced hyperemia Ach-induced hyperemia	Perfusion	AU; %-change	Perf.: 15 min. Anal.: 5 min. Perf.: 25–30 min. Anal.: 5 min. Perf.: 10–15 min. Anal.: 5 min.	Easy to perform; independent of skin pigmentation; measures functional reactivity	Indirect and relative measure of flow; mixed signal from arterioles and venules
Retinal photography	Microvessel: diameter; tortuosity; branching angle; fractal dimensions	AU	Perf.: 5 min. Anal.: diameters manually: 5–10 min; semiautomated: 1 min. Total set of variables automated: <1 min.	Direct visualization of arterioles, capillaries, venules; no mydriasis; easy to perform	Static single image: increased intra- and interindividual variability of vessel diameters (due to vasomotion)
Retinal videomicroscopy Flicker light–induced vasodilation		AU; %-change	Perf.: 5–10 min. Anal.: 2 min.	Direct visualization of arterioles, venules; measures functional reactivity	Mydriasis; requires good concentration/ compliance of participant

Perf., performance; art and ven occlusion, arterial and venous occlusion using a finger cuff; anal., analyses; ROI, region of interest; AU, arbitrary units.
[a]Usually 2–4 ROIs, in one or two fingers, are measured.

endothelial surface layer (glycocalyx) is important in the glomerular barrier function.[131,132] Endothelial activation leads to degradation of the glycocalyx with subsequent albuminuria, supporting the link between generalized endothelial activation, albuminuria, and renal/cardiometabolic disease.[1,132] Using sidestream darkfield imaging, it is now possible to measure glycocalyx dimensions of the sublingual microcirculation in a clinical setting.[133] For example, Dane *et al.*[133] showed that patients with ESRD had a thinner glycocalyx versus healthy controls, and glycocalyx thickness correlated with eGFR. Interestingly, glycocalyx thickness in patients with a stable kidney transplantation was found to be in-between that of patients with ESRD and controls, suggesting reversal of endothelial dysfunction.[133] Second, cerebral small vessel disease is a term used to describe pathologic, neuroimaging, and clinical features related to abnormalities of cerebral microvessels. Cerebral small vessel disease is associated with (incident) stroke, dementia, cognitive decline, and depression. With use of magnetic resonance imaging, various brain tissue abnormalities can be assessed (*e.g.*, white matter hyperintensities, microbleeds, lacunar infarcts) which indirectly reflect microcirculatory function. For further reading, please see references.[134,135] Third, near-infrared spectroscopy may be another interesting development. Near-infrared spectroscopy does not actually measure microvascular function, but measures O_2 delivery and tissue capacity to use O_2. Besides the skeletal muscle, this technique can also be applied to the brain, giving opportunities to study microcirculation-related end organ damage.[136,137] Future longitudinal and population-based studies are needed to prove the validity of these techniques in measuring microvascular function.

CONCLUSIONS

The studies reviewed here show that MVD is associated with many cardiometabolic/renal disease risk factors, and

precedes and contributes to the development of disease. MVD measured in different tissues tends to show similar associations with cardiometabolic risk factors, suggesting that common pathophysiologic mechanisms (*e.g.*, low grade inflammation, oxidative stress, *etc.*) are involved. It is, however, important to note that adaptation, to the same risk factor, may differ between vessel types. For example, cohort studies found BP to be inversely associated with retinal arteriolar but not, or even positively, with venular caliber.[138,139] In addition, in a cross-sectional study comparing microvascular responses to a mixed meal in obese versus lean individuals, Ach-induced skin arteriolar/venular vasodilation (measured with laser Doppler flowmetry) was attenuated in the obese, whereas skin capillary recruitment capacity was unchanged.[43]

Most of the studies reviewed have a cross-sectional design. Hence, longitudinal observational and intervention studies are needed to unravel how MVD contributes to the development and progression of disease. The technology to do so is now available. For individual risk assessment, normative data for each technique are needed across the sexes and age ranges, as are standardized protocols for measurement and analysis of data, preferably with automated investigator-independent software.

DISCLOSURES

None.

REFERENCES

1. Stehouwer CD, Smulders YM: Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. *J Am Soc Nephrol* 17: 2106–2111, 2006
2. Stehouwer CD: Is measurement of endothelial dysfunction clinically useful? *Eur J Clin Invest* 29: 459–461, 1999
3. Chew SK, Xie J, Wang JJ: Retinal arteriolar diameter and the prevalence and incidence of hypertension: A systematic review and meta-analysis of their association. *Curr Hypertens Rep* 14: 144–151, 2012
4. Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Tielsch JM, Klein BE, Hubbard LD: Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The sAtherosclerosis Risk in Communities study. *JAMA* 287: 1153–1159, 2002
5. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, Wofford MR, Sharrett AR: Retinal microvascular abnormalities and incident stroke: The Atherosclerosis Risk in Communities study. *Lancet* 358: 1134–1140, 2001
6. Yau JW, Xie J, Kawasaki R, Kramer H, Shlipak M, Klein R, Klein B, Cotch MF, Wong TY: Retinal arteriolar narrowing and subsequent development of CKD Stage 3: The Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis* 58: 39–46, 2011
7. Liew G, Mitchell P, Rochtchina E, Wong TY, Hsu W, Lee ML, Wainwright A, Wang JJ: Fractal analysis of retinal microvasculature and coronary heart disease mortality. *Eur Heart J* 32: 422–429, 2011
8. Mutlu U, Ikram MK, Wolters FJ, Hofman A, Klaver CC, Ikram MA: Retinal microvasculature is associated with long-term survival in the general adult dutch population. *Hypertension* 67: 281–287, 2016
9. Krentz AJ, Clough G, Byrne CD: Vascular disease in the metabolic syndrome: Do we need to target the microcirculation to treat large vessel disease? *J Vasc Res* 46: 515–526, 2009
10. Jacob M, Chappell D, Becker BF: Regulation of blood flow and volume exchange across the microcirculation. *Crit Care* 20: 319, 2016
11. Boulanger CM: Endothelium. *Arterioscler Thromb Vasc Biol* 36: e26–e31, 2016
12. Gutterman DD, Chabowski DS, Kadlec AO, Durand MJ, Freed JK, Ait-Aissa K, Beyer AM: The human microcirculation: Regulation of flow and beyond. *Circ Res* 118: 157–172, 2016
13. Pries AR, Secomb TW, Gaehtgens P: Structural autoregulation of terminal vascular beds: Vascular adaptation and development of hypertension. *Hypertension* 33: 153–161, 1999
14. Ley K: Leukocyte recruitment as seen by intravital microscopy. In: *Physiology of Inflammation*, 1st Ed., edited by Ley K, New York, Oxford University Press, 2001, pp 303–337
15. Aird WC: Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res* 100: 158–173, 2007
16. Chistiakov DA, Orekhov AN, Bobryshev YV: Effects of shear stress on endothelial cells: Go with the flow. *Acta Physiol (Oxf)* 219: 382–408, 2016
17. Aird WC: Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. *Circ Res* 100: 174–190, 2007
18. Domsic RT, Dezfulian C, Shoushtari A, Ivanco D, Kenny E, Kwoh CK, Medsger TA, Jr., Champion HC: Endothelial dysfunction is present only in the microvasculature and microcirculation of early diffuse systemic sclerosis patients. *Clin Exp Rheumatol* 32 [Suppl 86]: S-154–S-160, 2014
19. van Hecke MV, Dekker JM, Nijpels G, Stolk RP, Henry RM, Heine RJ, Bouter LM, Stehouwer CD, Polak BC: Are retinal microvascular abnormalities associated with large artery endothelial dysfunction and intima-media thickness? The Hoorn study. *Clin Sci (Lond)* 110: 597–604, 2006
20. Meyer MF, Lieps D, Schatz H, Pfohl M: Impaired flow-mediated vasodilation in type 2 diabetes: Lack of relation to microvascular dysfunction. *Microvasc Res* 76: 61–65, 2008
21. Serné 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* 38: 238–242, 2001
22. Gronenschild EH, Muris DM, Schram MT, Karaca U, Stehouwer CD, Houben AJ: Semi-automatic assessment of skin capillary density: Proof of principle and validation. *Microvasc Res* 90: 192–198, 2013
23. Serné EH, Stehouwer CD, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJ, Gans RO: Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 99: 896–902, 1999
24. Debbabi H, Uzan L, Mourad JJ, Safar M, Levy BI, Tibiriçà E: Increased skin capillary density in treated essential hypertensive patients. *Am J Hypertens* 19: 477–483, 2006
25. Irving RJ, Walker BR, Noon JP, Watt GC, Webb DJ, Shore AC: Microvascular correlates of blood pressure, plasma glucose, and insulin resistance in health. *Cardiovasc Res* 53: 271–276, 2002
26. Meijer RI, De Boer MP, Groen MR, Eringa EC, Rattigan S, Barrett EJ, Smulders YM, Serne EH: Insulin-induced microvascular recruitment in skin and muscle are related and both are associated with whole-body glucose uptake. *Microcirculation* 19: 494–500, 2012
27. Jonk AM, Houben AJ, Schaper NC, de Leeuw PW, Serné EH, Smulders YM, Stehouwer CD: Angiotensin II enhances insulin-stimulated whole-body glucose disposal but impairs insulin-induced capillary recruitment in healthy volunteers. *J Clin Endocrinol Metab* 95: 3901–3908, 2010
28. de Jongh RT, Serné EH, Ijzerman RG, de Vries G, Stehouwer CD: Free fatty acid levels modulate microvascular function: Relevance for obesity-associated insulin resistance, hypertension, and microangiopathy. *Diabetes* 53: 2873–2882, 2004
29. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ: Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes* 55: 1436–1442, 2006
30. 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* 89: 175–178, 2003
31. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA: Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension* 34: 655–658, 1999
32. Tibiriçá E, Rodrigues E, Cobas RA, Gomes MB: Endothelial function in patients with type 1 diabetes evaluated by skin capillary recruitment. *Microvasc Res* 73: 107–112, 2007
33. Thang OH, Serné EH, Grooteman MP, Smulders YM, ter Wee PM, Tangelder GJ, Nubé MJ: Capillary rarefaction in advanced chronic kidney disease is associated with high phosphorus and bicarbonate levels. *Nephrol Dial Transplant* 26: 3529–3536, 2011
34. Karatzi K, Protogerou A, Kesse-Guyot E, Fezeu LK, Carette C, Blacher J, Levy BI, Galan P, Hercberg S, Czernichow S: Associations between dietary patterns and skin microcirculation in healthy subjects. *Arterioscler Thromb Vasc Biol* 34: 463–469, 2014
35. Martens RJ, Henry RM, Houben AJ, van der Kallen CJ, Kroon AA, Schalkwijk CG, Schram MT, Sep SJ, Schaper NC, Dagnelie PC, Muris DM, Gronenschild EH, van der Sande FM, Leunissen KM, Kooman JP, Stehouwer CD: Capillary rarefaction associates with albuminuria: The Maastricht study. *J Am Soc Nephrol* 27: 3748–3757, 2016
36. de Boer MP, Meijer RI, Newman J, Stehouwer CD, Eringa EC, Smulders YM, Serné EH: Insulin-induced changes in microvascular vasomotion and capillary recruitment are associated in humans. *Microcirculation* 21: 380–387, 2014
37. Allen J, Howell K: Microvascular imaging: Techniques and opportunities for clinical physiological measurements. *Physiol Meas* 35: R91–R141, 2014
38. Fagrell B: The relationship between macro- and microcirculation clinical aspects. *Acta Pharmacol Toxicol (Copenh)* 58[Suppl 2]: 67–72, 1986
39. Thorn CE, Kyte H, Slaff DW, Shore AC: An association between vasomotion and oxygen extraction. *Am J Physiol Heart Circ Physiol* 301: H442–H449, 2011
40. Aalkjær C, Boedtkjer D, Matchkov V: Vasomotion - what is currently thought? *Acta Physiol (Oxf)* 202: 253–269, 2011
41. Schmidt-Lucke C, Borgström P, Schmidt-Lucke JA: Low frequency flowmotion/(vasomotion) during patho-physiological conditions. *Life Sci* 71: 2713–2728, 2002
42. Stefanovska A, Bracic M, Kvernmo HD: Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. *IEEE Trans Biomed Eng* 46: 1230–1239, 1999
43. Jonk AM, Houben AJ, Schaper NC, de Leeuw PW, Serné EH, Smulders YM, Stehouwer CD: Obesity is associated with impaired endothelial function in the postprandial state. *Microvasc Res* 82: 423–429, 2011
44. de Jongh RT, Clark AD, IJzerman RG, Serné EH, de Vries G, Stehouwer CD: Physiological hyperinsulinaemia increases intramuscular microvascular reactive hyperaemia and vasomotion in healthy volunteers. *Diabetologia* 47: 978–986, 2004
45. Rossi M, Bradbury A, Magagna A, Pesce M, Taddei S, Stefanovska A: Investigation of skin vasoreactivity and blood flow oscillations in hypertensive patients: Effect of short-term antihypertensive treatment. *J Hypertens* 29: 1569–1576, 2011
46. Rossi M, Carpi A, Galetta F, Franzoni F, Santoro G: Skin vasomotion investigation: A useful tool for clinical evaluation of microvascular endothelial function? *Biomed Pharmacother* 62: 541–545, 2008
47. Muris DM, Houben AJ, Kroon AA, Henry RM, van der Kallen CJ, Sep SJ, Koster A, Dagnelie PC, Schram MT, Stehouwer CD: Age, waist circumference, and blood pressure are associated with skin microvascular flow motion: The Maastricht study. *J Hypertens* 32: 2439–2449, discussion 2449, 2014
48. Agarwal SC, Allen J, Murray A, Purcell IF: Comparative reproducibility of dermal microvascular blood flow changes in response to acetylcholine iontophoresis, hyperthermia and reactive hyperaemia. *Physiol Meas* 31: 1–11, 2010
49. Choi PJ, Brunt VE, Fujii N, Minson CT: New approach to measure cutaneous microvascular function: An improved test of NO-mediated vasodilation by thermal hyperemia. *J Appl Physiol (1985)* 117: 277–283, 2014
50. Cracowski JL, Gaillard-Bigot F, Cracowski C, Sors C, Roustit M, Millet C: Involvement of cytochrome epoxygenase metabolites in cutaneous postocclusive hyperemia in humans. *J Appl Physiol (1985)* 114: 245–251, 2013
51. Hodges GJ, Sparks PA: Contributions of endothelial nitric oxide synthase, noradrenaline, and neuropeptide Y to local warming-induced cutaneous vasodilatation in men. *Microvasc Res* 90: 128–134, 2013
52. Henricson J, Tesselaar E, Persson K, Nilsson G, Sjöberg F: Assessment of microvascular function by study of the dose-response effects of iontophoretically applied drugs (acetylcholine and sodium nitroprusside)–methods and comparison with in vitro studies. *Microvasc Res* 73: 143–149, 2007
53. de Jongh RT, Serné EH, IJzerman RG, de Vries G, Stehouwer CD: Impaired microvascular function in obesity: Implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation* 109: 2529–2535, 2004
54. Czernichow S, Greenfield JR, Galan P, Bastard JP, Charnaux N, Samaras K, Safar ME, Blacher J, Hercberg S, Levy BI: Microvascular dysfunction in healthy insulin-sensitive overweight individuals. *J Hypertens* 28: 325–332, 2010
55. Montero D, Walther G, Perez-Martin A, Mercier CS, Gayrard S, Vicente-Salar N, Sempere-Ortells JM, Martinez-Peinado P, Roche E, Vinet A: Effects of a lifestyle program on vascular reactivity in macro- and microcirculation in severely obese adolescents. *J Clin Endocrinol Metab* 99: 1019–1026, 2014
56. Serné EH, Gans RO, ter Maaten JC, ter Wee PM, Donker AJ, Stehouwer CD: Capillary recruitment is impaired in essential hypertension and relates to insulin's metabolic and vascular actions. *Cardiovasc Res* 49: 161–168, 2001
57. Cupisti A, Rossi M, Placidi S, Fabbri A, Morelli E, Vagheggini G, Meola M, Barsotti G: Responses of the skin microcirculation to acetylcholine in patients with essential hypertension and in normotensive patients with chronic renal failure. *Nephron* 85: 114–119, 2000
58. Shah AS, Gao Z, Dolan LM, Dabelea D, D'Agostino RB, Jr., Urbina EM: Assessing endothelial dysfunction in adolescents and young adults with type 1 diabetes mellitus using a non-invasive heat stimulus. *Pediatr Diabetes* 16: 434–440, 2015
59. Heimhalt-El Hamriti M, Schreiver C, Noerenberg A, Scheffler J, Jacoby U, Haffner D, Fischer DC: Impaired skin microcirculation in paediatric patients with type 1 diabetes mellitus. *Cardiovasc Diabetol* 12: 115, 2013
60. Brooks BA, McLennan SV, Twigg SM, Yue DK: Detection and characterisation of microcirculatory abnormalities in the skin of diabetic patients with microvascular complications. *Diab Vasc Dis Res* 5: 30–35, 2008
61. Stansberry KB, Hill MA, Shapiro SA, McNitt PM, Bhatt BA, Vinik AI: Impairment of peripheral blood flow responses in diabetes resembles an enhanced aging effect. *Diabetes Care* 20: 1711–1716, 1997
62. Rathsman B, Jensen-Urstad K, Nyström T: Intensified insulin treatment is associated with improvement in skin microcirculation and ischaemic foot ulcer in patients with type 1 diabetes mellitus: A long-term follow-up study. *Diabetologia* 57: 1703–1710, 2014
63. Sörensen BM, Houben AJ, Berendschot TT, Schouten JS, Kroon AA, van der Kallen CJ, Henry RM, Koster A, Sep SJ, Dagnelie PC, Schaper NC, Schram MT, Stehouwer CD: Prediabetes and type 2 diabetes are associated with generalized microvascular dysfunction: The Maastricht study. *Circulation* 134: 1339–1352, 2016

64. Settergren M, Böhm F, Rydén L, Pernow J, Kalani M: Lipid lowering versus pleiotropic effects of statins on skin microvascular function in patients with dysglycaemia and coronary artery disease. *J Intern Med* 266: 492–498, 2009
65. Fronek A, DiTomasso DG, Allison M: Noninvasive assessment of endothelial activity in patients with peripheral arterial disease and cardiovascular risk factors. *Endothelium* 14: 199–205, 2007
66. Kruger A, Stewart J, Sahityani R, O'Riordan E, Thompson C, Adler S, Garrick R, Vallance P, Goligorsky MS: Laser Doppler flowmetry detection of endothelial dysfunction in end-stage renal disease patients: Correlation with cardiovascular risk. *Kidney Int* 70: 157–164, 2006
67. Houben AJ, Schaper NC, Slaaf DW, Tangelder GJ, Nieuwenhuijzen Kruseman AC: Skin blood cell flux in insulin-dependent diabetic subjects in relation to retinopathy or incipient nephropathy. *Eur J Clin Invest* 22: 67–72, 1992
68. Schmiedel O, Schroeter ML, Harvey JN: Microalbuminuria in Type 2 diabetes indicates impaired microvascular vasomotion and perfusion. *Am J Physiol Heart Circ Physiol* 293: H3424–H3431, 2007
69. Lim SC, Caballero AE, Smakowski P, LoGerfo FW, Horton ES, Veves A: Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. *Diabetes Care* 22: 1865–1870, 1999
70. Thang OH, Serné EH, Grooteman MP, Smulders YM, Ter Wee PM, Tangelder GJ, Nubé MJ: Premature aging of the microcirculation in patients with advanced chronic kidney disease. *Nephron Extra* 2: 283–292, 2012
71. Rossi M, Cupisti A, Di Maria C, Galetta F, Barsotti G, Santoro G: Blunted post-ischemic increase of the endothelial skin blood flow-motion component as early sign of endothelial dysfunction in chronic kidney disease patients. *Microvasc Res* 75: 315–322, 2008
72. Hughes AD, Wong TY, Witt N, Evans R, Thom SA, Klein BE, Chaturvedi N, Klein R: Determinants of retinal microvascular architecture in normal subjects. *Microcirculation* 16: 159–166, 2009
73. Azemin MZ, Kumar DK, Wong TY, Wang JJ, Mitchell P, Kawasaki R, Wu H: Age-related rarefaction in the fractal dimension of retinal vessel. *Neurobiol Aging* 33: 194.e1–194.e4, 2012
74. Sun C, Wang JJ, Mackey DA, Wong TY: Retinal vascular caliber: Systemic, environmental, and genetic associations. *Surv Ophthalmol* 54: 74–95, 2009
75. Lehmann MV, Schmieder RE: Remodeling of retinal small arteries in hypertension. *Am J Hypertens* 24: 1267–1273, 2011
76. Dorner GT, Garhofer G, Kiss B, Polska E, Polak K, Riva CE, Schmetterer L: Nitric oxide regulates retinal vascular tone in humans. *Am J Physiol Heart Circ Physiol* 285: H631–H636, 2003
77. Delles C, Michelson G, Harazny J, Oehmer S, Hilgers KF, Schmieder RE: Impaired endothelial function of the retinal vasculature in hypertensive patients. *Stroke* 35: 1289–1293, 2004
78. Kiss B, Polska E, Dorner G, Polak K, Findl O, Mayrl GF, Eichler HG, Wolzt M, Schmetterer L: Retinal blood flow during hyperoxia in humans revisited: Concerted results using different measurement techniques. *Microvasc Res* 64: 75–85, 2002
79. Leung H, Wang JJ, Rochtchina E, Wong TY, Klein R, Mitchell P: Impact of current and past blood pressure on retinal arteriolar diameter in an older population. *J Hypertens* 22: 1543–1549, 2004
80. Sharrett AR, Hubbard LD, Cooper LS, Sorlie PD, Brothers RJ, Nieto FJ, Pinsky JL, Klein R: Retinal arteriolar diameters and elevated blood pressure: The Atherosclerosis Risk in Communities study. *Am J Epidemiol* 150: 263–270, 1999
81. Murgan I, Beyer S, Kotliar KE, Weber L, Bechtold-Dalla Pozza S, Dalla Pozza R, Wegner A, Sitnikova D, Stock K, Heemann U, Schmaderer C, Baumann M: Arterial and retinal vascular changes in hypertensive and prehypertensive adolescents. *Am J Hypertens* 26: 400–408, 2013
82. Ding J, Wai KL, McGeechan K, Ikram MK, Kawasaki R, Xie J, Klein R, Klein BB, Cotch MF, Wang JJ, Mitchell P, Shaw JE, Takamasa K, Sharrett AR, Wong TY; Meta-Eye Study Group: Retinal vascular caliber and the development of hypertension: A meta-analysis of individual participant data. *J Hypertens* 32: 207–215, 2014
83. Kaushik S, Wang JJ, Flood V, Liew G, Smith W, Mitchell P: Frequency of fish consumption, retinal microvascular signs and vascular mortality. *Microcirculation* 15: 27–36, 2008
84. Antonio PR, Marta PS, Luís DD, Antonio DP, Manuel ST, Rafael MS, Sonia GV, Manuel GP, Isabel MN, Carlos EN, Gabriel CT, Francisco GU: Factors associated with changes in retinal microcirculation after antihypertensive treatment. *J Hum Hypertens* 28: 310–315, 2014
85. Hughes AD, Stanton AV, Jabbar AS, Chapman N, Martinez-Perez ME, McG Thom SA: Effect of antihypertensive treatment on retinal microvascular changes in hypertension. *J Hypertens* 26: 1703–1707, 2008
86. Hanssen H, Nickel T, Drexel V, Hertel G, Emslander I, Sisic Z, Lorang D, Schuster T, Kotliar KE, Pressler A, Schmidt-Trucksäss A, Weis M, Halle M: Exercise-induced alterations of retinal vessel diameters and cardiovascular risk reduction in obesity. *Atherosclerosis* 216: 433–439, 2011
87. Sabanayagam C, Shankar A, Koh D, Chia KS, Saw SM, Lim SC, Tai ES, Wong TY: Retinal microvascular caliber and chronic kidney disease in an Asian population. *Am J Epidemiol* 169: 625–632, 2009
88. Baumann M, Burkhardt K, Heemann U: Microcirculatory marker for the prediction of renal end points: A prospective cohort study in patients with chronic kidney disease stage 2 to 4. *Hypertension* 64: 338–346, 2014
89. Sabanayagam C, Shankar A, Klein BE, Lee KE, Muntner P, Nieto FJ, Tsai MY, Cruickshanks KJ, Schubert CR, Brazy PC, Coresh J, Klein R: Bidirectional association of retinal vessel diameters and estimated GFR decline: The Beaver Dam CKD study. *Am J Kidney Dis* 57: 682–691, 2011
90. Metea MR, Newman EA: Signalling within the neurovascular unit in the mammalian retina. *Exp Physiol* 92: 635–640, 2007
91. Nagel E, Vilser W, Lanzl I: Age, blood pressure, and vessel diameter as factors influencing the arterial retinal flicker response. *Invest Ophthalmol Vis Sci* 45: 1486–1492, 2004
92. Kotliar KE, Lanzl IM, Schmidt-Trucksäss A, Sitnikova D, Ali M, Blume K, Halle M, Hanssen H: Dynamic retinal vessel response to flicker in obesity: A methodological approach. *Microvasc Res* 81: 123–128, 2011
93. Al-Fiadh AH, Wong TY, Kawasaki R, Clark DJ, Patel SK, Freeman M, Wilson A, Burrell LM, Farouque O: Usefulness of retinal microvascular endothelial dysfunction as a predictor of coronary artery disease. *Am J Cardiol* 115: 609–613, 2015
94. Heitmar R, Varma C, De P, Lau YC, Blann AD: The relationship of systemic markers of renal function and vascular function with retinal blood vessel responses. *Graefes Arch Clin Exp Ophthalmol* 254: 2257–2265, 2016
95. 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* 118: 968–976, 2008
96. Noon JP, Walker BR, Webb DJ, Shore AC, Holton DW, Edwards HV, Watt GC: Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. *J Clin Invest* 99: 1873–1879, 1997
97. O'Connor DT, Tyrell EA, Kailasam MT, Miller LM, Martinez JA, Henry RR, Parmer RJ, Gabbai FB: Early alteration in glomerular reserve in humans at genetic risk of essential hypertension: Mechanisms and consequences. *Hypertension* 37: 898–906, 2001
98. Gluckman PD, Hanson MA, Cooper C, Thornburg KL: Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 359: 61–73, 2008

99. Touwslager RN, Houben AJ, Gielen M, Zeegers MP, Stehouwer CD, Zimmermann LJ, Kessels AG, Gerver WJ, Blanco CE, Mulder AL: Endothelial vasodilatation in newborns is related to body size and maternal hypertension. *J Hypertens* 30: 124–131, 2012
100. Martin H, Gazelius B, Norman M: Impaired acetylcholine-induced vascular relaxation in low birth weight infants: Implications for adult hypertension? *Pediatr Res* 47: 457–462, 2000
101. 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* 58: 847–851, 2011
102. Bonamy AK, Martin H, Jörneskog G, Norman M: Lower skin capillary density, normal endothelial function and higher blood pressure in children born preterm. *J Intern Med* 262: 635–642, 2007
103. Lewandowski AJ, Davis EF, Yu G, Digby JE, Boardman H, Whitworth P, Singhal A, Lucas A, McCormick K, Shore AC, Leeson P: Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. *Hypertension* 65: 607–614, 2015
104. Hellström A, Dahlgren J, Marsál K, Ley D: Abnormal retinal vascular morphology in young adults following intrauterine growth restriction. *Pediatrics* 113: e77–e80, 2004
105. Liew G, Wang JJ, Duncan BB, Klein R, Sharrett AR, Brancati F, Yeh HC, Mitchell P, Wong TY; Atherosclerosis Risk in Communities Study: Low birthweight is associated with narrower arterioles in adults. *Hypertension* 51: 933–938, 2008
106. Luyckx VA, Brenner BM: The clinical importance of nephron mass. *J Am Soc Nephrol* 21: 898–910, 2010
107. Montero D, Walther G, Diaz-Cañestro C, Pyke KE, Padilla J: Microvascular dilator function in thletes: A Systematic Review and Meta-analysis. *Med Sci Sports Exerc* 47: 1485–1494, 2015
108. Lanting SM, Johnson NA, Baker MK, Caterson ID, Chuter VH: The effect of exercise training on cutaneous microvascular reactivity: A systematic review and meta-analysis *J Sci Med Sport* 20: 170–177, 2016
109. Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, Gokce N, Ruderman NB, Keaney JF, Jr., Vita JA: Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol* 27: 2650–2656, 2007
110. Demiot C, Dignat-George F, Fortrat JO, Sabatier F, Gharib C, Larina I, Gauquelin-Koch G, Hughson R, Custaud MA: WISE 2005: Chronic bed rest impairs microcirculatory endothelium in women. *Am J Physiol Heart Circ Physiol* 293: H3159–H3164, 2007
111. Tikellis G, Anuradha S, Klein R, Wong TY: Association between physical activity and retinal microvascular signs: The Atherosclerosis Risk in Communities (ARIC) study. *Microcirculation* 17: 381–393, 2010
112. Anuradha S, Dunstan DW, Healy GN, Shaw JE, Zimmet PZ, Wong TY, Owen N: Physical activity, television viewing time, and retinal vascular caliber. *Med Sci Sports Exerc* 43: 280–286, 2011
113. Thijssen DH, Green DJ, Hopman MT: Blood vessel remodeling and physical inactivity in humans. *J Appl Physiol (1985)* 111: 1836–1845, 2011
114. Gielen S, Schuler G, Adams V: Cardiovascular effects of exercise training: Molecular mechanisms. *Circulation* 122: 1221–1238, 2010
115. Robinson ES, Fisher ND, Forman JP, Curhan GC: Physical activity and albuminuria. *Am J Epidemiol* 171: 515–521, 2010
116. Wadén J, Tikkanen HK, Forsblom C, Harjutsalo V, Thorn LM, Saraheimo M, Tolonen N, Rosengård-Bärlund M, Gordin D, Tikkanen HO, Groop PH; FinnDiane Study Group: Leisure-time physical activity and development and progression of diabetic nephropathy in type 1 diabetes: The FinnDiane study. *Diabetologia* 58: 929–936, 2015
117. Jonk AM, Houben AJ, de Jongh RT, Semé EH, Schaper NC, Stehouwer CD: Microvascular dysfunction in obesity: A potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Physiology (Bethesda)* 22: 252–260, 2007
118. Siegrist M, Hanssen H, Neidig M, Fuchs M, Lechner F, Stetten M, Blume K, Lammel C, Haller B, Vogeser M, Parhofer KG, Halle M: Association of leptin and insulin with childhood obesity and retinal vessel diameters. *Int J Obes* 38: 1241–1247, 2014
119. Boillot A, Zoungas S, Mitchell P, Klein R, Klein B, Ikram MK, Klaver C, Wang JJ, Gopinath B, Tai ES, Neubauer AS, Hercberg S, Brazionis L, Saw SM, Wong TY, Czernichow S; META-EYE Study Group: Obesity and the microvasculature: A systematic review and meta-analysis. *PLoS One* 8: e52708, 2013
120. Shankar A, Sabanayagam C, Klein BE, Klein R: Retinal microvascular changes and the risk of developing obesity: Population-based cohort study. *Microcirculation* 18: 655–662, 2011
121. Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ: Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. *Diabetes Care* 32: 1672–1677, 2009
122. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD: Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 97: 2601–2610, 1996
123. Scalia R: The microcirculation in adipose tissue inflammation. *Rev Endocr Metab Disord* 14: 69–76, 2013
124. Eringa EC, Bakker W, van Hinsbergh VW: Paracrine regulation of vascular tone, inflammation and insulin sensitivity by perivascular adipose tissue. *Vascul Pharmacol* 56: 204–209, 2012
125. Eringa EC, Serne EH, Meijer RI, Schalkwijk CG, Houben AJ, Stehouwer CD, Smulders YM, van Hinsbergh VW: Endothelial dysfunction in (pre)diabetes: Characteristics, causative mechanisms and pathogenic role in type 2 diabetes. *Rev Endocr Metab Disord* 14: 39–48, 2013
126. Zhang X, Lerman LO: Obesity and renovascular disease. *Am J Physiol Renal Physiol* 309: F273–F279, 2015
127. Kanasaki K, Kitada M, Kanasaki M, Koya D: The biological consequence of obesity on the kidney. *Nephrol Dial Transplant* 28 [Suppl 4]: iv1–iv7, 2013
128. Myers CE, Klein R, Knudtson MD, Lee KE, Gangnon R, Wong TY, Klein BE: Determinants of retinal venular diameter: The Beaver Dam Eye study. *Ophthalmology* 119: 2563–2571, 2012
129. Denic A, Lieske JC, Chakkera HA, Poggio ED, Alexander MP, Singh P, Kremers WK, Lerman LO, Rule AD: The substantial loss of nephrons in healthy human kidneys with aging. *J Am Soc Nephrol* 28: 313–320, 2017
130. El Assar M, Angulo J, Rodríguez-Mañas L: Oxidative stress and vascular inflammation in aging. *Free Radic Biol Med* 65: 380–401, 2013
131. Satchell SC, Tooke JE: What is the mechanism of microalbuminuria in diabetes: A role for the glomerular endothelium? *Diabetologia* 51: 714–725, 2008
132. Rabelink TJ, de Zeeuw D: The glycocalyx–linking albuminuria with renal and cardiovascular disease. *Nat Rev Nephrol* 11: 667–676, 2015
133. Dane MJ, Khairoun M, Lee DH, van den Berg BM, Eskens BJ, Boels MG, van Teeffelen JW, Rops AL, van der Vlag J, van Zonneveld AJ, Reinders ME, Vink H, Rabelink TJ: Association of kidney function with changes in the endothelial surface layer. *Clin J Am Soc Nephrol* 9: 698–704, 2014
134. Østergaard L, Engedal TS, Moreton F, Hansen MB, Wardlaw JM, Dalkara T, Markus HS, Muir KW: Cerebral small vessel disease: Capillary pathways to stroke and cognitive decline. *J Cereb Blood Flow Metab* 36: 302–325, 2016
135. Wardlaw JM, Smith C, Dichgans M: Mechanisms of sporadic cerebral small vessel disease: Insights from neuroimaging. *Lancet Neurol* 12: 483–497, 2013
136. Kainerstorfer JM, Sassaroli A, Tgavalekos KT, Fantini S: Cerebral autoregulation in the microvasculature measured with near-infrared

spectroscopy. *J Cereb Blood Flow Metab* 35: 959–966, 2015
137. Hamaoka T, McCully KK, Quaresima V, Yamamoto K, Chance B: Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *J Biomed Opt* 12: 062105, 2007
138. 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* 29: 1380–1391, 2011
139. Kumagai K, Tabara Y, Yamashiro K, Miyake M, Akagi-Kurashige Y, Oishi M, Yoshikawa M, Kimura Y, Tsujikawa A, Takahashi Y, Setoh K, Kawaguchi T, Terao C, Yamada R, Kosugi S, Sekine A, Nakayama T, Matsuda F, Yoshimura N; Nagahama Study group: Central blood pressure relates more strongly to retinal arteriolar narrowing than brachial blood pressure: The Nagahama study. *J Hypertens* 33: 323–329, 2015