

# Assessing Microvascular Function in Humans from a Chronic Disease Perspective

Alfons J.H.M. Houben,<sup>\*†</sup> Remy J.H. Martens,<sup>\*‡</sup> and Coen D.A. Stehouwer<sup>\*†</sup>

<sup>\*</sup>Department of Internal Medicine, Maastricht University Medical Center+, Maastricht, The Netherlands; and <sup>†</sup>CARIM School for Cardiovascular Diseases and <sup>‡</sup>School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands

## 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.<sup>1,2</sup>

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<sup>3–5</sup> and renal disease,<sup>6</sup> and is associated with increased (cardiovascular) mortality.<sup>7,8</sup> MVD often coexists with or even precedes macrovascular disease, possibly due to shared mechanisms of vascular damage.<sup>9</sup> A key player in MVD is the endothelium.<sup>10,11</sup> 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.*<sup>12</sup> 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  $\mu$ m 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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**Correspondence:** Dr. Alfons J.H.M. Houben, Department of Internal Medicine, Maastricht University Medical Center+, P. Debyelaan 25, PO Box 5800, 6202 AZ Maastricht, The Netherlands. Email: [b.houben@maastrichtuniversity.nl](mailto:b.houben@maastrichtuniversity.nl)

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of control of vascular resistance.<sup>13</sup> 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.<sup>14,15</sup>

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.<sup>15–17</sup> 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.<sup>17</sup> 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)<sup>18–20</sup> may be related to differences in endothelial phenotype in the different parts of the vascular tree.

### Capillary Microscopy

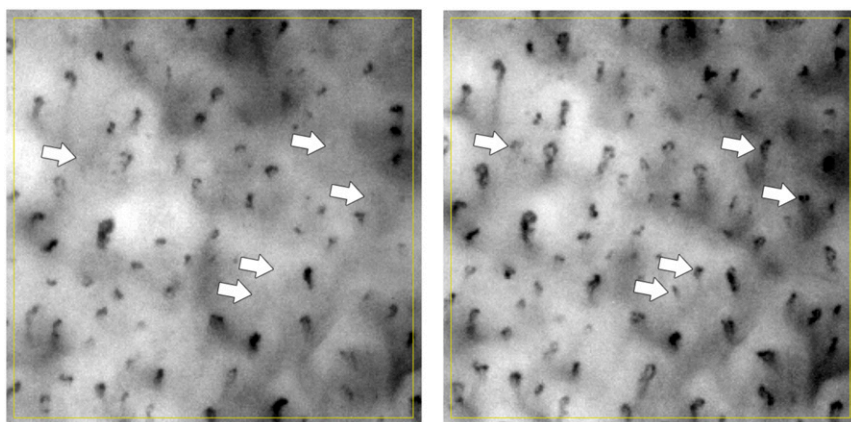
Skin is a unique site for simple and reproducible assessment of capillary structure and function, where intravital capillaroscopy can be used to directly visualize perfused nutritive capillaries. At the finger and toe nailfold, capillaries run in parallel to the skin surface, which enables evaluation 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 $\times$ . 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<sup>21,22</sup> (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.<sup>23–25</sup> 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,<sup>26,27</sup> whereas the presence of obesity or increased free fatty acid levels attenuates capillary recruitment.<sup>28,29</sup> 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.<sup>30,31</sup> In more advanced disease, such as type 2 diabetes, hypertension, and advanced CKD, capillary rarefaction is also seen.<sup>21,32,33</sup> In a healthy cohort (mean age approximately 62 $\pm$ 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.<sup>34</sup> In addition, in a population-based study (mean age approximately 60 $\pm$ 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.<sup>35</sup> 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<sup>36</sup> 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<sup>3</sup> 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<sup>2</sup> 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.<sup>37</sup> The laser-Doppler signal comes predominantly from small arterioles and venules, and to a lesser extent from capillaries.<sup>38</sup>

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,<sup>39,40</sup> and reduces hydraulic resistance.<sup>41</sup> 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.<sup>42</sup> 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,<sup>43,44</sup> and that these reactions are diminished in obesity. In untreated hypertensive subjects, flowmotion is augmented and normalizes after treatment of hypertension.<sup>45</sup> 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.<sup>46</sup> 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.<sup>47</sup> 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.<sup>48</sup> Both postocclusive and heat-induced reactive hyperemia are partly endothelium dependent<sup>49,50</sup> (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.<sup>51,52</sup> 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.<sup>23,25</sup> In cross-sectional studies, the Ach-response has been found to be

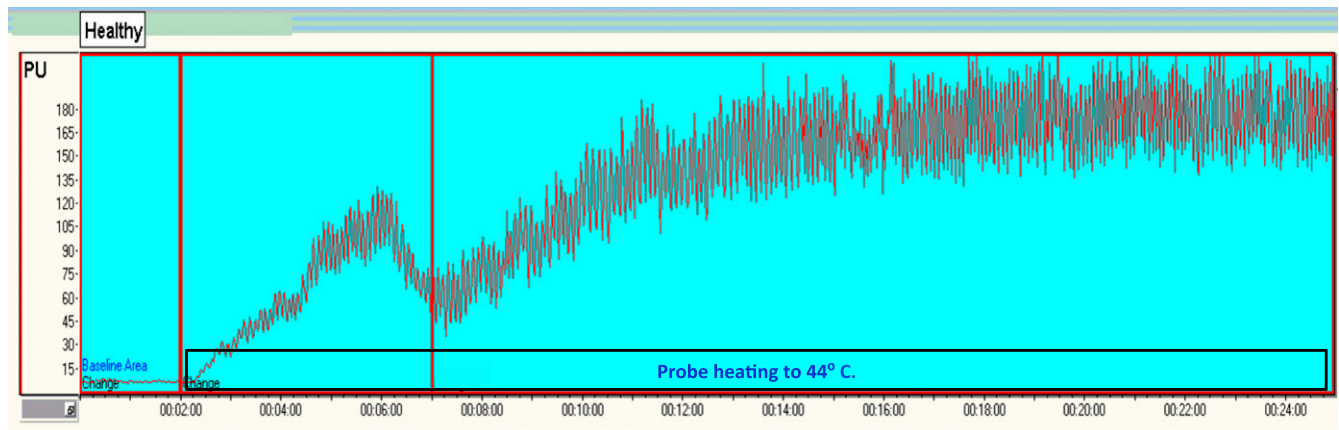
reduced in adults with obesity,<sup>53</sup> but not in obese adolescents or overweight adults.<sup>54,55</sup> Hypertensive, as compared with normotensive, individuals also show a reduced Ach-response.<sup>56,57</sup> Several studies on both type 1 and 2 diabetes have shown reduced Ach- and heat-induced vasodilation, which is worse when complications are present.<sup>58–61</sup> These vasodilator responses are inversely related to the level of glycemic control, and improve with intensified glucose control.<sup>58,62</sup> 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.<sup>63</sup> 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.<sup>64–66</sup>

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

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



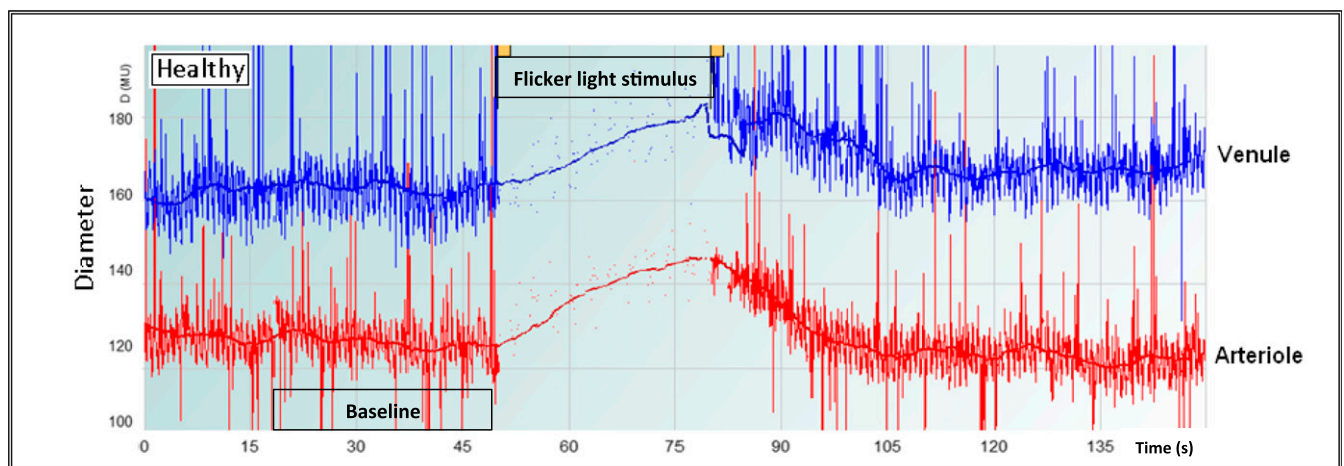
**Figure 2.** Typical registration of skin microvascular perfusion, measured with laser-Doppler flowmetry, before and during local heating of the skin in a healthy volunteer. After 2 minutes of baseline flow registration, skin heating to 44°C is started for 23 minutes. Time (minute) is depicted on the 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.<sup>72,73</sup> Mechanisms of changes in retinal vessel diameters can be both functional and structural.<sup>74,75</sup> For arterioles this involves changes in endothelial vasodilators (*e.g.*,

NO)<sup>76</sup> and constrictors, and BP-related remodeling of the vessel wall.<sup>74,75</sup> For venular widening, inflammatory signals and endothelial dysfunction have been suggested to be involved.<sup>74</sup> 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<sup>63,76–78</sup> (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.<sup>79–81</sup> Smaller arterioles may not only be an adaptive response to higher BP, but also predict (and possibly contribute to) the development of hypertension.<sup>82</sup> 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.<sup>83</sup> In addition, 6–12 months of BP treatment resulted in wider arteriolar diameters.<sup>84,85</sup> Finally, lifestyle interventions may also be a treatment option to normalize microvascular diameters.<sup>86</sup>

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<sup>2</sup> or the presence of micro/macroalbuminuria) independent of the presence of diabetes and hypertension.<sup>87</sup> 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.<sup>88</sup>

However, longitudinal population-based cohort studies did not find associations between baseline retinal arteriolar/venular diameters and incident CKD.<sup>6,89</sup>

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.<sup>76,90</sup> 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.<sup>63</sup> In small cross-sectional studies, similar findings of reduced retinal endothelial function have been found in individuals with hypertension, obesity, and coronary artery disease.<sup>91–93</sup> Recently, it was shown, in patients with diabetes and/or cardiovascular disease, that retinal endothelial function correlates with creatinine clearance and eGFR.<sup>94</sup> Because follow-up studies on retinal vasoreactivity are

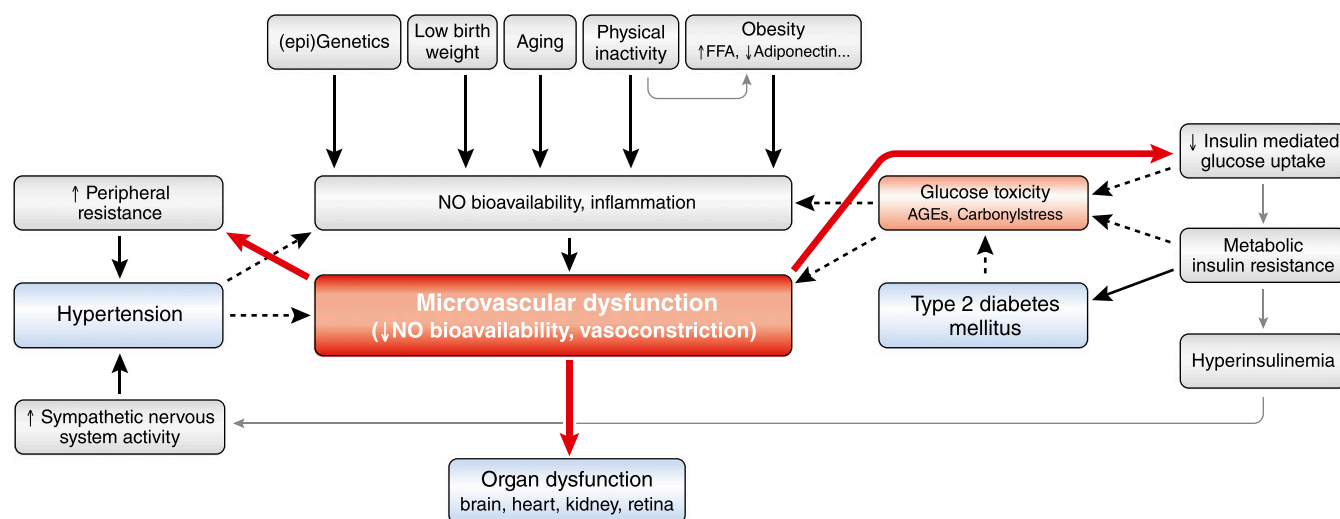
scarce, the prognostic value of these measurements remains to be explored. Nevertheless, microvascular (endothelial) reactivity data add valuable (patho)physiologic information to static retinal diameter/morphometry data.

## 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,<sup>95</sup> with a lower number of skin capillaries (rarefaction) and reduced capillary recruitment capacity as compared with matched control individuals.<sup>30</sup> In another study,<sup>96</sup> 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.<sup>97</sup> 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.<sup>98</sup> 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.<sup>99,100</sup> In contrast, functional and structural skin capillary densities seem to be higher in low birth weight as compared with normal birth weight newborns,<sup>101</sup> although at prepubertal age this seems to be reversed.<sup>102</sup> In addition, adults who were born preterm show reduced skin capillary densities,<sup>103</sup> and retinal arteriolar diameters and vascularization.<sup>104,105</sup>

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.<sup>106</sup>

### Physical Inactivity

Two meta-analyses have shown that endothelium-(in)dependent microvascular function is enhanced in both athletes and trained adults versus healthy controls.<sup>107,108</sup> Vice versa, physical inactivity has been found to induce MVD acutely in healthy volunteers after bed rest.<sup>109,110</sup> In addition, population-

based cohort studies have shown that less physical activity/increased television viewing time is associated with wider retinal venular diameters.<sup>111,112</sup> 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.<sup>113,114</sup> 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.<sup>115</sup> 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.<sup>116</sup>

### Obesity

Many studies have shown that MVD is present in obesity.<sup>117</sup> Already at a young age, obesity is independently associated with smaller retinal arteriolar and wider venular diameters,<sup>118</sup> which continues in adults.<sup>119</sup> Wider retinal venular, but not smaller arteriolar, diameters may predict development of obesity.<sup>120</sup> In addition, skin capillary recruitment capacity<sup>53</sup> and impaired endothelium-dependent microvascular dilation in skin and muscle have been found in obese individuals.<sup>43,121,122</sup> Several mechanisms may be involved in obesity-related MVD. Elevated free fatty acid levels augment skin MVD,<sup>28</sup> 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).<sup>117,123</sup> 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.<sup>124</sup>

Relevant clinical consequences of obesity-related MVD are insulin resistance and raised BP.<sup>117</sup> Subsequently, chronic hyperglycemia contributes to further deterioration of microvascular endothelial function.<sup>125</sup> Raised BP contributes to endothelial dysfunction, arteriolar wall remodeling, and capillary/arteriolar rarefaction.<sup>95</sup> 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.<sup>126</sup> The latter likely contributes to the development of secondary FSGS and loss of kidney function in individuals with (severe) obesity.<sup>127</sup>

### 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.<sup>128</sup> In another cohort, age was independently associated with skin microvascular flowmotion.<sup>47</sup> These findings in the systemic microcirculation parallel the significant loss of nephrons with aging observed in healthy kidney donors.<sup>129</sup> Oxidative stress and inflammatory processes in the endothelium have been proposed to be the main drivers of MVD in aging.<sup>130</sup>

### 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 <sup>2</sup> ; % change	Perf.: baseline, art and ven occlusion: approximately 11 min. Anal.: 15 min per RO <sup>13</sup> /finger (semiautomated) <sup>22</sup> ; manually: 30 min.	Direct visualization of capillaries; measures functional reactivity	Difficult in dark skin; laborious analyses
Skin laser-doppler flowmetry	Perfusion	AU; % change	Perf.: 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
Heat-induced hyperemia			Perf.: 25–30 min.		
Ach-induced hyperemia			Perf.: 5 min. Anal.: 5 min.		
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; RO<sup>13</sup>, region of interest; AU, arbitrary units.

<sup>a</sup>Usually 2–4 ROIs, in one or two fingers, are measured.

endothelial surface layer (glycocalyx) is important in the glomerular barrier function.<sup>131,132</sup> Endothelial activation leads to degradation of the glycocalyx with subsequent albuminuria, supporting the link between generalized endothelial activation, albuminuria, and renal/cardiometabolic disease.<sup>1,132</sup> Using side-stream darkfield imaging, it is now possible to measure glycocalyx dimensions of the sublingual microcirculation in a clinical setting.<sup>133</sup> For example, Dane *et al.*<sup>133</sup> 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.<sup>133</sup> 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.<sup>134,135</sup> Third, near-infrared spectroscopy may be another interesting development. Near-infrared spectroscopy does not actually measure microvascular function, but measures O<sub>2</sub> delivery and tissue capacity to use O<sub>2</sub>. Besides the skeletal muscle, this technique can also be applied to the brain, giving opportunities to study microcirculation-related end organ damage.<sup>136,137</sup> 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.<sup>138,139</sup> 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.<sup>43</sup>

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.

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