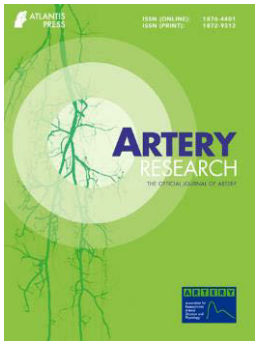

Artery Research

ISSN (Online): 1876-4401 **ISSN (Print): 1872-9312**
Journal Home Page: https://www.atlantis-press.com/journals/artres

The metabolic-microvascular dysregulation syndrome☆

Coen D.A. Stehouwer

To cite this article: Coen D.A. Stehouwer (2018) The metabolic-microvascular dysregulation syndrome☆, Artery Research 21:C, 78–83, DOI: https://doi.org/10.1016/j.artres.2017.12.005

To link to this article: https://doi.org/10.1016/j.artres.2017.12.005

Published online: 3 December 2019

Artery Research (2018) 21, 78–83

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/artres

The metabolic-microvascular dysregulation syndrome☆

Coen D.A. Stehouwer

Department of Internal Medicine, Maastricht University Medical Centre+, 6202 AZ Maastricht, The Netherlands

Received 12 December 2017; accepted 14 December 2017
Available online 10 January 2018

KEYWORDS
Microcirculation;
Microvascular function;
Endothelium;
Metabolism;
Hyperglycaemia;
Insulin resistance;
Obesity

Introduction

The McDonald Lecture honours Donald Arthur McDonald (1917-1973), a British physiologist who established the modern approach to the study of arterial haemodynamics over a 20-year period from 1953-1973. His work established the logic of using Fourier analysis to break down pressure and flow waves, and developed the general concept of vascular impedance.[1] His classic book on blood flow in arteries was published in 1960,[2] and has remained a basic treatise in this field for more than 50 years.[3] Linking with engineers and with physicians, he influenced many young physicians and physiologists. He directed work into the clinical sphere while continuing in basic physiology and haemodynamics.[1] Donald McDonald thus is an intellectual godfather of the ARTERY society, and I am honoured to present the 2017 McDonald Lecture.

ARTERY's goal is to promote the advancement of knowledge and dissemination of information concerning the pathophysiology, pharmacology, epidemiology, detection, investigation and treatment of arterial structure and function. Thus its goal is to further the understanding of human diseases from the point of view of large artery structure and function. As a clinical scientist, I submit that understanding human disease is impossible without crossing borders, notably that from large arteries to the microcirculation. Indeed, the society's journal, *Artery Research*, publishes papers not only in the classic domain of arterial structure and function and its interaction with various organs such as the heart, kidney and brain, but has on occasion published papers that focus entirely on the microcirculation.[4,5]

The microcirculation is widely taken to encompass vessels <150 μm in diameter. It therefore includes arterioles, capillaries, and venules. A definition based on arterial vessel physiology rather than diameter or structure has also

☆ Presented as the invited McDonald Lecture at the Artery 17 Meeting of the Association for Research into Arterial Structure and Physiology, Pisa, Italy, 12–14 October, 2017.

E-mail address: cda.stehouwer@mumc.nl.

https://doi.org/10.1016/j.artres.2017.12.005

been proposed, depending on the response of the isolated vessel to increased internal pressure. By this definition, all vessels that respond to increasing pressure with a myogenic reduction in lumen diameter are considered part of the microcirculation, including the smallest arteries and arterioles in addition to capillaries and venules. A primary function of the microcirculation is *metabolic*, to optimize the delivery of nutrients and removal of waste products in response to variations in demand. A second important function is to avoid large fluctuations in hydrostatic pressure at the level of the capillaries that otherwise would impair capillary exchange. Finally, it is at the level of the microcirculation that a substantial proportion of the drop in hydrostatic pressure occurs. The microcirculation is, therefore, extremely important in determining the overall peripheral resistance. In normal conditions, systemic, regional, and local metabolic and myogenic autoregulatory mechanisms ensure adequate progress of these microcirculatory functions. In pathological conditions, however, the loss of such mechanisms results in the development of microvascular dysfunction.[6]

At the ARTERY14 meeting, in 2014, Alun Hughes elegantly considered the design of the circulation (large and small vessels) from the point of view of optimality and cost minimization.[7] In this McDonald Lecture, I shall take this one step further and develop *the concept that microvascular and metabolic physiology are inextricably linked*. Indeed, I shall postulate that dysfunction of the one causes dysfunction of the other, justifying the concept of a 'Metabolic-Microvascular Dysregulation Syndrome'.

Microvascular consequences of metabolic dysregulation

Diabetic retinopathy is the classic example of the link between metabolism and microvessels. In diabetic retinopathy, metabolic dysregulation (hyperglycaemia) causes many microvascular changes, such as microaneurysms, haemorrhages, and hard and soft exudates.[8] There is convincing evidence that the link is causal; thus, reduction of hyperglycaemia reduces onset and progression of retinopathy.[9,10]

Diabetic nephropathy is a second example. Morphologically, diabetic nephropathy is less exclusively microvascular than is diabetic retinopathy, and is characterized by arteriolar hyalinosis but most typically by glomerular basement membrane thickening and mesangial expansion.[11] Nevertheless, microvascular endothelial dysfunction has been shown to be a core feature of diabetic nephropathy. Functionally, there is first an increase in glomerular filtration rate (hyperfiltration), followed by a steady decrease over time. In parallel, urinary albumin excretion increases from normal (<30 mg/24h) to microalbuminuria (30-300 mg/24h) and macroalbuminuria (>300 mg/24h).[12] It is in the urinary leakage of albumin that microvascular endothelial dysfunction is especially important. A key observation, in the 1980s and 1990s, was that in type 2 diabetes, type 1 diabetes and in the general population even a slight increase in urinary albumin excretion (microalbuminuria) was associated with a large increase in risk of cardiovascular events.[13-15] This association has in many studies been shown to be independent of conventional risk factors and persists over many years.[16] From renal physiology it follows that excess albuminuria must be explained by excess permeation across the glomerular capillary wall (itself dependent on glomerular pressure, permeability and surface area) and/or impaired renal tubular reabsorption.[17] Cell types involved include podocytes, microvascular (arteriolar and glomerular) endothelial cells, mesangial cells and tubular cells. Of these, endothelial cells, if dysfunctional not only in the kidney but also elsewhere, could potentially explain the link between microalbuminuria and cardiovascular disease (i.e., atherothrombosis).

In late 1980s, we set out to investigate this concept with then available markers of microvascular endothelial dysfunction, such as plasma levels of von Willebrand factor and, later, of adhesion molecules (sVCAM-1, sICAM-1, sE-selectin). We found that levels of such biomarkers were strongly associated with onset and progression of microalbuminuria in type 1 and type 2 diabetes as well as in the general population,[18-20] as reviewed elsewhere.[21] It took many years to develop more direct methods to investigate microvascular endothelial function at the population level.[22] Studies using such methods further supported the concept of microalbuminuria as a marker of microvascular endothelial dysfunction. In the Maastricht Study,[23] albuminuria was associated with capillary density (assessed in skin),[24] heat-induced microvascular dilation (also assessed in skin), and flicker-light-induced arteriolar dilation (assessed in the retina) (Martens et al, unpublished observations).

Taken together, these findings establish that relatively severe hyperglycaemia, as in diabetes, can cause microvascular disease (retinopathy and albuminuria). We recently showed that less severe hyperglycaemia (prediabetes) is also associated with microvascular dysfunction. The association between glycaemia and microvascular dysfunction in fact appears not to have a threshold and can be demonstrated in skin[25,26], retina[25,26] and brain, in the latter as white matter hyperintensities (Van Agtmaal et al, unpublished observations), which are thought to represent cerebral small vessel disease.

Microvascular dysfunction demonstrated with such methods is clinically relevant. For example, biomarkers of microvascular dysfunction are associated with depression both cross-sectionally and longitudinally;[27,28] microalbuminuria is associated not only with myocardial infarction and stroke but also with depression[27,28] and impairment of cognitive function;[29] and white matter hyperintensities predict stroke, dementia, depression and mortality (Rensma et al, unpublished observations).

Taken together, there is a continuous, presumably causal, association between glycaemia and microvascular dysfunction, which does not have a clear threshold, and which predisposes to clinical disease.

Metabolic consequences of microvascular dysregulation

Observations over the past 25 years have convincingly shown that microvascular dysregulation impairs normal

metabolism.[6] Insulin is an endothelium-dependent vasodilator.[30] In animal models, insulin can recruit previously underperfused nutritive capillary networks in skeletal muscle.[31-33] This action of insulin on the microcirculation enhances insulin-mediated glucose disposal and indeed is necessary for normal insulin sensitivity; conversely, microvascular endothelial dysfunction impairs insulin-mediated glucose disposal, i.e. causes insulin resistance.[7,33] Using skin as a model, we were the first to show that insulin has similar microvascular actions in humans[34-36] and that insulin-induced microvascular recruitment contributes to glucose disposal independently of visceral, subcutaneous and intrahepatic fat.[37] Interestingly, an animal model in which insulin signal transduction is selectively impaired in endothelial cells (and normal everywhere else) shows not only impaired insulin-induced microvascular dilation and whole-body glucose disposal,[38] but also impaired insulin secretion,[39] suggesting that normal endothelial function is needed for normal glucose-induced insulin secretion. This has yet to be demonstrated in humans.

The studies reviewed above show that normal microvascular endothelial function is necessary both for normal insulin-mediated glucose disposal and for glucose-induced insulin secretion. It logically follows that impaired microvascular endothelial function should predispose to the development of type 2 diabetes. We have found that indeed to be the case, regardless of how microvascular dysfunction was measured.[40] Thus, the association between hyperglycaemia and microvascular endothelial dysfunction is bidirectional. This constitutes the core of the Metabolic-Microvascular Dysregulation Syndrome.

Obesity as a driver of the metabolic-microvascular dysregulation syndrome (Fig. 1)

Obesity is associated with microvascular dysfunction, including impaired insulin-induced microvascular dilation and recruitment.[37,41-43] Normal insulin action in endothelial cells is to increase synthesis of nitric oxide through the PI3 kinase pathway more than endothelin synthesis through the ERK pathway, with vasodilation as the net result.[44-46]

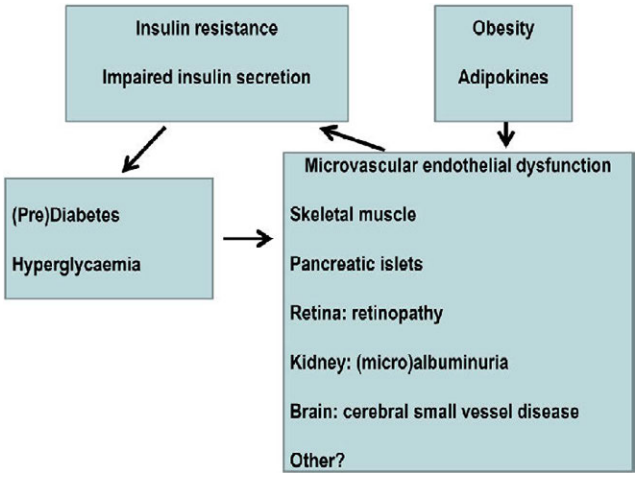

Figure 1 Overview of the Metabolic-Microvascular Dysregulation Syndrome.

Obesity shifts this balance towards less vasodilation or even vasoconstriction through adverse changes in adipokines such as adiponectin, free fatty acids and tumour necrosis factor-α, which impair insulin signal transduction in endothelial cells to impair nitric oxide synthesis and/or enhance endothelin production.[47-53] For example, in lean humans, an acute increase in FFA levels impairs microvascular recruitment in response to reactive hyperaemia and insulin; conversely, in obese humans, an overnight decrease in FFA levels induced by acipimox increases microvascular recruitment in response to reactive hyperaemia and insulin.[54] These adipokines may derive from not only visceral fat but also from subcutaneous (truncal) and perivascular fat.[37,53,55]

Microvascular dysfunction in obesity appears reversible. In abdominally obese men, diet-induced weight loss ($\sim$10 kg in eight weeks), as compared to a control group receiving a weight-maintenance diet, improved whole-body glucose disposal in part through improvement of insulin-induced microvascular recruitment in skeletal muscle.[37] Abdominal obesity was also associated with a lower retinal arteriole-to-venule ratio, which normalised after weight loss.[56] In summary, obesity is associated with widespread microvascular dysfunction, which is at least partly reversible on weight loss.[37,56]

Perspectives

Obesity is a major factor in setting up the syndrome, but other factors are also likely to play a role. I shall briefly discuss three of these. Firstly, and fascinatingly, the hypothesis has been advanced that microvascular dysfunction of adipose tissue is a primary cause of adipose tissue dysfunction resulting in adverse changes in adipokines.[57] These findings[57] raise the possibility that microvascular dysfunction is at the very core of the Metabolic-Microvascular Dysregulation Syndrome. Secondly, early life exposures, both antenatal and postnatal, are likely to be important. For example, in otherwise healthy individuals born at term, low birth weight is associated with impaired microvascular function, both in adults[58] and in prepubertal children.[59] In healthy newborns, smaller size at birth and maternal hypertension were associated with impaired microvascular endothelial function;[60] and rapid growth in the first month was inversely associated with microvascular endothelial function at six months of age.[61] This observation suggests that rapid growth can be detrimental to microvascular endothelial function. In this respect, the early postnatal period may be a critical period and provide a window of opportunity for prevention of microvascular endothelial dysfunction, for example through nutritional means.[61] Finally, what of arterial stiffening? Stiffening of large arteries impairs their cushioning function and increases pressure and flow pulsatility, which transmits distally and can damage the microcirculation.[62,63] Indeed, previous studies have shown an association between greater arterial stiffness and markers of microvascular dysfunction in the brain (cerebral small vessel lesions),[64] eye (retinal arteriolar narrowing),[65] and kidney (microalbuminuria).[66,67] These organs are especially vulnerable to the detrimental effects of increased pressure and flow pulsatility, as their microvasculature is characterized by low impedance, allowing the

pulsatile load to penetrate deeply into their microvascular beds.[62,63] In contrast, there is no strong evidence that other organs are similarly affected. For example, we have not found significant associations between aortic or carotid stiffness and plasma biomarkers of microvascular endothelial dysfunction[68,69] or an array of estimates of skin microvascular function.[70] Thus, the microcirculation of most organs may be able to protect itself against the detrimental effects of increased arterial stiffness and pressure and flow pulsatility through relatively high microvascular impedance as a result of effective autoregulation and/or vascular remodelling. This would dissipate most of the increased pulsatile energy by arteries and large arterioles proximal to capillary beds and thus limit penetration of the pulsatile load. In sum, large artery stiffening is unquestionably important for microvascular function in susceptible organs such as the brain, the eye and the kidney, but there is no clear evidence that this is a generalized phenomenon. It is therefore not clear that arterial stiffening in and of itself is sufficient to cause the Metabolic-Microvascular Dysregulation Syndrome. It should be kept in mind, however, that associations between arterial stiffening and microcirculatory function in organs that are probably essential for setting up the syndrome, such skeletal muscle, pancreas and adipose tissue, have yet to be examined.

Conclusions

Microvascular and metabolic physiology are inextricably linked. I here propose that dysfunction of the one causes dysfunction of the other, justifying the concept of a 'Metabolic-Microvascular Dysregulation Syndrome'. For example, metabolic dysregulation (hyperglycaemia) causes microvascular dysfunction, diabetic retinopathy and diabetic nephropathy. Conversely, microvascular dysregulation impairs insulin-mediated glucose disposal, i.e. causes insulin resistance, impairs insulin secretion, and is associated with onset of type 2 diabetes in prospective studies. Obesity is a key driver of the Metabolic-Microvascular Dysregulation Syndrome, as it impairs insulin signal transduction in endothelial cells through adverse changes in adipokines such as adiponectin, free fatty acids and tumour necrosis factor-α. Microvascular dysfunction in obesity appears reversible by diet-induced weight loss. Next to obesity, other factors are also likely to play a role. Examples are microvascular dysfunction of adipose tissue as a primary cause of adipose tissue dysfunction; early life exposures, both antenatal and postnatal; and large artery stiffening. Large artery stiffening is unquestionably important for microvascular function in susceptible organs such as the brain, the eye and the kidney but whether it can cause microvascular dysfunction in metabolically crucial tissues such as skeletal muscle, pancreas and adipose tissue has not been studied. It is therefore not clear that arterial stiffening in and of itself is sufficient to cause the Metabolic-Microvascular Dysregulation Syndrome.

References

1. O'Rourke MF, Taylor MT. Profiles in cardiology: Donald Arthur McDonald. *Clin Cardiol* 1993;**16**:842—3.
2. McDonald DA. *Blood flow in arteries*. London: Arnold; 1960.
3. Nichols WW, O'Rourke MF, Vlachopoulos C. *McDonald's blood flow in arteries: theoretical, experimental and clinical principles*. 6th ed. London: Hodder Arnold;; 2011.
4. Liu YP, Richart T, Jin Y, Struijker-Boudier HA, Staessen JA. Retinal arteriolar and venular phenotypes in a Flemish population: reproducibility and correlates. *Artery Res* 2011;**5**:72—9.
5. Aissopou EK, Protogerou AD, Papaioannou TG, Tektonidou M, Tentolouris N, Theodossiadis PG, et al. Retinal vascular calibers in contemporary patients with chronic systemic inflammatory diseases: the Greek REtinal Microcirculation (GREM) study. *Artery Res* 2017;**18**:1—6.
6. Jonk AM, Houben AJHM, de Jongh RT, Serné EH, Schaper NC, Stehouwer CDA. Microvascular dysfunction in obesity: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Physiology* 2007;**22**:252—60.
7. Hughes AD. Optimality, cost minimization and the design of arterial networks. *Artery Res* 2015;**10**:1—10.
8. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. *Lancet* 2010;**376**:124—36.
9. Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;**329**:977—86.
10. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998;**352**:837—53.
11. Fioretto P, Mauer M. Histopathology of diabetic nephropathy. *Semin Nephrol* 2007;**27**:195—207.
12. Mogensen CE. Prediction of clinical diabetic nephropathy in IDDM patients. Alternatives to microalbuminuria? *Diabetes* 1990;**39**:761—7.
13. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 1984;**310**:356—60.
14. Rossing P, Hougaard P, Borch-Johnsen K, Parving HH. Predictors of mortality in insulin dependent diabetes: 10 year observational follow up study. *BMJ* 1996;**313**:779—84.
15. Damsgaard EM, Frøland A, Jørgensen OD, Mogensen CE. Eight to nine year mortality in known non-insulin dependent diabetics and controls. *Kidney Int* 1992;**41**:731—5.
16. Beijers HJ, Ferreira I, Bravenboer B, Dekker JM, Nijpels G, Heine RJ, Stehouwer CD. Microalbuminuria and cardiovascular autonomic dysfunction are independently associated with cardiovascular mortality: evidence for distinct pathways: the Hoorn Study. *Diabetes Care* 2009;**32**:1698—703.
17. D'Amico G, Bazzi C. Pathophysiology of proteinuria. *Kidney Int* 2003;**63**:809—25.
18. Stehouwer CDA, Stroes ESG, Hackeng WHL, Mulder PGH, den Ottolander GJH. Von Willebrand Factor and development of diabetic nephropathy in insulin-dependent diabetes mellitus. *Diabetes* 1991;**40**:971—6 [erratum, Diabetes 1991;40:1746].
19. Stehouwer CDA, Nauta JJP, Zeldenrust GC, Hackeng WHL, Donker AJM, den Ottolander GJH. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dependent diabetes mellitus. *Lancet* 1992;**340**:319—23.
20. Stehouwer CDA, Fischer HRA, van Kuijk AWR, Polak BCP, Donker AJM. Endothelial dysfunction precedes the development of microalbuminuria in insulin-dependent diabetes mellitus. *Diabetes* 1995;**44**:561—4.
21. Stehouwer CDA, Smulders YM. Microalbuminuria and risk of cardiovascular disease: analysis of potential mechanisms. *J Am Soc Nephrol* 2006;**17**:2106—11.
22. Houben AJHM, Martens RJH, Stehouwer CDA. Assessing microvascular function in humans from a chronic disease perspective. *J Am Soc Nephrol* 2017;**28**:3461—72.

23. Schram MT, Sep SJS, van der Kallen CJH, Dagnelie PC, Koster A, Schaper N, et al. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. *Eur J Epidem* 2014;**29**:439—51.
24. Martens RJH, Henry RMA, Houben AJHM, van der Kallen CJH, Kroon AA, Schalkwijk CG, et al. Capillary rarefaction associates with albuminuria: the Maastricht Study. *J Am Soc Nephrol* 2016;**27**:3748—57.
25. Sörensen BM, Houben AJHM, Berendschot TTJM, Schouten JSAG, Kroon AA, van der Kallen CJH, et al. Prediabetes and type 2 diabetes are associated with generalized microvascular dysfunction: the Maastricht Study. *Circulation* 2016;**134**:1339—52.
26. Sörensen BM, Houben AJHM, Berendschot TTJM, Schouten JSAG, Kroon AA, van der Kallen CJH, et al. Hyperglycemia is the main mediator of prediabetes- and type 2 diabetes-associated impairment of microvascular function: the Maastricht Study. *Diabetes Care* 2017;**40**:e103—5.
27. van Agtmaal MJM, Houben AJHM, Pouwer F, Stehouwer CDA, Schram MT. Association of microvascular dysfunction with late-life depression: a systematic review and meta-analysis. *JAMA Psychiatry* 2017;**74**:729—39.
28. Martens RJ, Kooman JP, Stehouwer CD, Dagnelie PC, van der Kallen CJ, Kroon AA, et al. Albuminuria is associated with a higher prevalence of depression in a population-based cohort study: the Maastricht Study. *Nephrol Dial Transplant* 2017. https://doi.org/10.1093/ndt/gfw377 [Epub ahead of print].
29. Martens RJ, Kooman JP, Stehouwer CD, Dagnelie PC, van der Kallen CJ, Koster A, et al. Estimated GFR, albuminuria, and cognitive performance: the Maastricht Study. *Am J Kidney Dis* 2017;**69**:179—91.
30. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest* 1990;**85**:1844—52.
31. Newman JM, Dora KA, Rattigan S, Edwards SJ, Colquhoun EQ, Clark MG. Norepinephrine and serotonin vasoconstriction in rat hindlimb control different vascular flow routes. *Am J Physiol* 1996;**270**(4 Pt 1):E689—99.
32. Rattigan S, Clark MG, Barrett EJ. Acute vasoconstriction-induced insulin resistance in rat muscle in vivo. *Diabetes* 1999;**48**:564—9.
33. Clark MG, Wallis MG, Barrett EJ, Vincent MA, Richards SM, Clerk LH, et al. Blood flow and muscle metabolism: a focus on insulin action. *Am J Physiol Endocrinol Metab* 2003;**284**:E241—58.
34. Serné EH, Stehouwer CDA, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJM, et al. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. *Circulation* 1999;**99**:896—902.
35. Serné EH, Gans ROB, Maaten JC, ter Wee PM, Donker AJM, Stehouwer CDA. Capillary recruitment is impaired in essential hypertension and relates to insulin's metabolic and vascular actions. *Cardiovasc Res* 2001;**49**:161—8.
36. Serné EH, IJzerman RG, Gans ROB, Nijveldt R, de Vries G, Evertz R, et al. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. *Diabetes* 2002;**51**:1515—22.
37. Kusters YH, Schalkwijk CG, Houben AJ, Kooi ME, Lindeboom L, Op 't Roodt J, et al. Independent tissue contributors to obesity-associated insulin resistance. *JCI Insight* 2017. https://doi.org/10.1172/jci.insight.89695 [Epub ahead of print].
38. Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, et al. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 2011;**13**:294—307.
39. Hashimoto S, Kubota N, Sato H, Sasaki M, Takamoto I, Kubota T, et al. Insulin receptor substrate-2 (Irs2) in endothelial cells plays a crucial role in insulin secretion. *Diabetes* 2015;**64**:876—86.
40. Muris DMJ, Houben AJHM, Schram MT, Stehouwer CDA. Microvascular dysfunction is associated with a higher incidence of type 2 diabetes mellitus: a systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol* 2012;**32**:3082—94.
41. de Jongh RT, Serné EH, IJzerman RG, de Vries G, Stehouwer CDA. Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation* 2004;**109**:2529—35.
42. de Jongh RT, IJzerman RG, Serné EH, Voordouw JJ, Yudkin JS, Delemarre-van de Waal HA, et al. Visceral and truncal subcutaneous adipose tissue are associated with impaired capillary recruitment in healthy individuals. *J Clin Endocrinol Metab* 2006;**91**:5100—6.
43. de Jongh RT, Serné EH, IJzerman RG, Jørstad HT, Stehouwer CDA. Impaired local microvascular vasodilatory effects of insulin and reduced skin microvascular vasomotion in obese women. *Microvasc Res* 2008;**75**:256—62.
44. Zeng G, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest* 1996;**98**:894—8.
45. Cardillo C, Nambi SS, Kilcoyne CM, Choucair WK, Katz A, Quon MJ, et al. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation* 1999;**100**:820—5.
46. Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, et al. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 2003;**111**:1373—80.
47. Eringa EC, Stehouwer CDA, Merlijn T, Westerhof N, Sipkema P. Physiological concentrations of insulin induce endothelin-mediated vasoconstriction during inhibition of NOS or PI3-Kinase in skeletal muscle arterioles. *Cardiovasc Res* 2002;**56**:464—71.
48. Eringa EC, Stehouwer CDA, van Nieuw Amerongen GP, Ouwehand L, Westerhof N, Sipkema P. Vasoconstrictor effects of insulin in skeletal muscle arterioles are mediated by ERK1/2 activation in endothelium. *Am J Physiol Heart Circ Physiol* 2004;**287**:H2043—8.
49. Eringa EC, Stehouwer CDA, Walburg K, Clark AD, van Nieuw Amerongen GP, Westerhof N, et al. Physiological concentrations of insulin induce endothelin-dependent vasoconstriction of skeletal muscle resistance arteries in the presence of tumor necrosis factor-α: dependence on c-jun N-terminal kinase. *Arterioscler Thromb Vasc Biol* 2006;**26**:274—80.
50. Eringa EC, Stehouwer CDA, Roos MH, Westerhof N, Sipkema P. Selective resistance to vasoactive effects of insulin in muscle resistance arteries of obese Zucker (fa/fa) rats. *Am J Physiol Endocrinol Metab* 2007;**293**:E1134—9.
51. Bakker W, Sipkema P, Stehouwer CDA, Serné EH, Smulders YM, van Hinsbergh VWM, Eringa EC. Protein kinase C theta activation induces insulin-mediated constriction of muscle resistance arteries. *Diabetes* 2008;**57**:706—13.
52. Bradley EA, Eringa EC, Stehouwer CDA, Korstjens I, van Nieuw Amerongen GP, Musters R, et al. Activation of AMP-activated kinase by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside in the muscle microcirculation increases NO synthesis and microvascular perfusion. *Arterioscler Thromb Vasc Biol* 2010;**30**:1137—42.
53. Meijer RI, Bakker W, Alta CL, Sipkema P, Yudkin JS, Viollet B, et al. Perivascular adipose tissue control of insulin-induced vasoreactivity in muscle is impaired in db/db mice. *Diabetes* 2013;**62**:590—8.
54. de Jongh RT, Serné EH, IJzerman RG, de Vries G, Stehouwer CDA. Free fatty acid levels modulate microvascular function: relevance for obesity-associated insulin resistance,

hypertension, and microangiopathy. *Diabetes* 2004;**53**:2873—82.
55. Yudkin JS, Eringa EC, Stehouwer CDA. Vasocrine signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet* 2005;**365**:1817—20.
56. Joris PJ, Plat J, Kusters YH, Houben AJ, Stehouwer CD, Schalkwijk CG, et al. Diet-induced weight loss improves not only cardiometabolic risk markers but also markers of vascular function: a randomized controlled trial in abdominally obese men. *Am J Clin Nutr* 2017;**105**:23—31.
57. Robciuc MR, Kivelä R, Williams IM, de Boer JF, van Dijk TH, Elamaa H, et al. VEGFB/VEGFR1-induced expansion of adipose vasculature counteracts obesity and related metabolic complications. *Cell Metab* 2016;**23**:712—24.
58. Serné EH, Stehouwer CDA, ter Maaten JC, ter Wee PM, Donker AJM, Gans ROB. Birth weight relates to blood pressure and microvascular function in normal subjects. *J Hypertens* 2000;**18**:1421—7.
59. IJzerman RG, van Weissenbruch MM, Voordouw JJ, Yudkin JS, Delemarre-van de Waal H, Stehouwer CDA. The association between birth weight and capillary recruitment is independent of blood pressure and insulin sensitivity: a study in prepubertal children. *J Hypertens* 2002;**20**:1957—63.
60. Touwslager RNH, Houben AJHM, Gielen M, Zeegers MP, Stehouwer CDA, Zimmermann LJ, et al. Endothelial function in newborns is related to body size and maternal hypertension. *J Hypertens* 2012;**30**:124—31.
61. Touwslager RNH, Gerver WJM, Tan FES, Gielen M, Zeegers MP, Zimmermann LJ, et al. Influence of growth during infancy on endothelium-dependent vasodilatation at the age of 6 months. *Hypertension* 2012;**60**:1294—300.
62. Mitchell GF. Effects of central arterial aging on the structure and function of the peripheral vasculature: implications for end-organ damage. *J Appl Physiol* 1985;**2008**(105):1652—60.
63. O'Rourke MF, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension* 2005;**46**:200—4.
64. Mitchell GF, van Buchem MA, Sigurdsson S, Gotal JD, Jonsdottir MK, Kjartansson Ó, et al. Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility—Reykjavik study. *Brain* 2011;**134**:3398—407.
65. Cheung N, Sharrett AR, Klein R, Criqui MH, Islam FM, Macura KJ, et al. Aortic distensibility and retinal arteriolar narrowing: the multi-ethnic study of atherosclerosis. *Hypertension* 2007;**50**:617—22.
66. Hermans MMH, Henry RMA, Dekker JM, Kooman JP, Kostense PJ, Nijpels G, et al. Estimated glomerular filtration rate and urinary albumin excretion are independently associated with greater arterial stiffness — the Hoorn Study. *J Am Soc Nephrol* 2007;**18**:1942—52.
67. Briet M, Boutouyrie P, Laurent S, London GM. Arterial stiffness and pulse pressure in CKD and ESRD. *Kidney Int* 2012;**82**:388—400.
68. van Bussel BCT, Schouten F, Henry RMA, Schalkwijk CG, de Boer MR, Ferreira I, et al. Endothelial dysfunction and low-grade inflammation are associated with greater arterial stiffness over a 6-year period — The Amsterdam Growth and Health Longitudinal Study. *Hypertension* 2011;**58**:588—95.
69. van Bussel BCT, Henry RMA, Schalkwijk CG, Ferreira I, Dekker JM, Nijpels G, et al. Low-grade inflammation, but not endothelial dysfunction, is associated with greater carotid stiffness in the elderly — the Hoorn Study. *J Hypertens* 2012;**30**:744—52.
70. van Sloten TT, Czernichow S, Houben AJ, Protogerou AD, Henry RMA, Muris DM, et al. Association between arterial stiffness and skin microvascular function: the SUVIMAX2 Study and the Maastricht Study. *Am J Hypertens* 2015;**28**:868—76.