

SYMPOSIUM

Vasculoprotective properties of the endothelial glycocalyx: effects of fluid shear stress

M. GOUVERNEUR¹, B. VAN DEN BERG², M. NIEUWDORP³, E. STROES³ & H. VINK¹

From the ¹Department of Medical Physics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, ²Department of Molecular and Vascular Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA, and ³Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Abstract. Gouverneur M, van den Berg B, Nieuwdorp M, Stroes E, Vink H. (University of Amsterdam, Amsterdam, the Netherlands; and Harvard Medical School, Boston, MA, USA). Vasculoprotective properties of the endothelial glycocalyx: effects of fluid shear stress *J Intern Med* 2006; **259**: 393–400.

The endothelial glycocalyx exerts a wide array of vasculoprotective effects via inhibition of coagulation and leucocyte adhesion, by contributing to the vascular permeability barrier and by mediating shear stress-induced NO release. In this review, we

will focus on the relationship between fluid shear stress and the endothelial glycocalyx. We will address the hypothesis that modulation of glycocalyx synthesis by fluid shear stress may contribute to thinner glycocalyces, and therefore more vulnerable endothelium, at lesion-prone sites of arterial bifurcations. Finally, we will discuss the effects of known atherogenic stimuli such as hyperglycaemia on whole body glycocalyx volume in humans and its effect on endothelial function.

Keywords: atherosclerosis, glycocalyx, hyaluronan, shear stress.

Introduction

Cardiovascular disease is the major cause of mortality worldwide and notwithstanding many efforts to reduce cardiovascular disease burden, current strategies aimed at lowering systemic risk factors have only achieved a 20–30% reduction in cardiovascular event rate [1]. The remaining 70–80% of events highlights the need for novel strategies to improve cardiovascular outcome. The insight that all cardiovascular risk factors inflict loss of anti-atherogenic properties of the vessel wall, has shifted attention from only treating systemic risk factors towards augmenting vasculoprotective properties of the vessel wall itself. As the endothelium is the first-

line defence mechanism against atherosclerosis, much research effort has focused at novel strategies to improve endothelial function.

Over the past several years, it is recognized that the endothelial glycocalyx may contribute to the protection of the vascular wall against disease. The glycocalyx, consisting of a negatively charged, organized mesh of membranous glycoproteins, proteoglycans, glycosaminoglycans (GAGs) and associated plasma proteins, is situated at the luminal side of all blood vessels [2]. Its major constituents comprise hyaluronic acid and the negatively charged heparan sulphate proteoglycans. Glycocalyx dimensions depend upon the balance between biosynthesis and enzymatic or shear-dependent

shedding of its components [3], and whereas historically this layer was thought to be confined to a thickness of only several nanometres, it has recently been demonstrated to reach up to 0.5–3 μm intraluminally [4, 5]. This relatively large dimension of the glycocalyx, which exceeds the thickness of the endothelium and the length of leucocyte adhesion molecules, has triggered researchers to study its role in the course of atherogenesis [6].

Numerous studies in both micro- and macrovasculature have demonstrated that constituents of the glycocalyx, such as hyaluronan, are intimately involved in vascular homeostasis, such as maintaining the vascular permeability barrier [7] and regulating the release of nitric oxide (NO) by serving as a mechano-shear sensor for NO release [8–11]. In addition, the glycocalyx harbours a wide array of enzymes that might contribute to its vasculoprotective effect. Thus, extracellular superoxide dismutase (ec-SOD), an enzyme which dismutates oxygen radicals to hydrogen peroxide [12], is bound to proteoglycans within the glycocalyx. Damage to the glycocalyx is accompanied by increased shedding of ec-SOD, which results in a dysbalance in favour of a pro-oxidant state [13]. Collectively, these observations are of particular interest as altered vascular permeability, attenuated NO-bioavailability and redox dysregulation are amongst the earliest characteristics of atherogenesis [14]. In spite of these observations, it has proved difficult to show direct relevance of the glycocalyx as a vasculoprotective paradigm for larger vessels. The latter is predominantly due to the fact that glycocalyx research has traditionally focused at the microvasculature, in which atherogenesis does not occur.

Structural properties of the endothelial glycocalyx

The first visualization of the endothelial glycocalyx was performed by conventional electron microscopy using the cationic dye ruthenium red, which has a high affinity for acidic mucopolysaccharides [15]. Electron micrographs revealed a small irregular shaped layer extending approximately 50–100 nm into the vessel lumen. Subsequent approaches with varying perfusate contents or fixatives revealed stained structures on continuous endothelial cell surfaces throughout diverse microvascular beds,

arterial- and venular macrovessels with large variations in dimension and appearance [4, 16–20]. Fenestrated endothelium, in addition, was found to have a combination of surface-bound stained structures, about 50–100 nm thick, and distinct filamentous plugs composed of 20 to 40 filaments with a length of about 350 nm on the surface of the endothelial fenestrae [21]. These studies, especially when specific approaches were applied to stabilize anionic carbohydrate structures to prevent loss- and or collapse of these structures, gave evidence for a thick endothelial surface layer throughout the whole vascular tree (Fig. 1). In addition, co-localization of lectins to the observed stained structures confirmed its saccharine nature in several of these studies [4, 16, 18].

Intravital microscopy studies on cremaster muscle showed dramatic differences between microvascular- and systemic haematocrit [22] that could be abrogated upon enzymatic treatment of the microvascular network with heparinase [23]. From these studies a 0.3–1 μm thick slow-moving plasma layer on the endothelial cell surface consisting principally of heparan-sulphate proteoglycans was thought to be involved. The first visual evidence of a 0.4–0.5 μm thick continuous endothelial cell surface layer was provided by comparing the width of the flowing plasma column containing large, anionic fluorescein-labelled dextrans with the anatomic capillary diameter as defined by the position of the luminal endothelial cell boundaries [24]. Based on observations in this study, theoretical studies predicted a glycocalyx thickness of 0.5–1 μm to account for observed variations in red-cell motion through microvessels and the discrepancy between *in vivo* and *in vitro* estimates of resistance to blood flow [25–27]. Indeed, such differences in blood flow resistance have been observed between control and hyaluronidase-treated vessels in a study of coronary reactive hyperaemia in a dog [28]. Enzymatic degradation of the glycocalyx with hyaluronidase has been shown to significantly increase the available intraluminal space for flowing blood [7].

Although various studies are consistent with the concept that perturbation of the glycocalyx contributes to increases in endothelial vulnerability upon ischaemia/reperfusion [17], hypoxia [20], exposure to low-density lipoproteins [29, 30] and atherogenic shear stress profiles [6, 18], it has proved difficult to show direct relevance of the glycocalyx as a

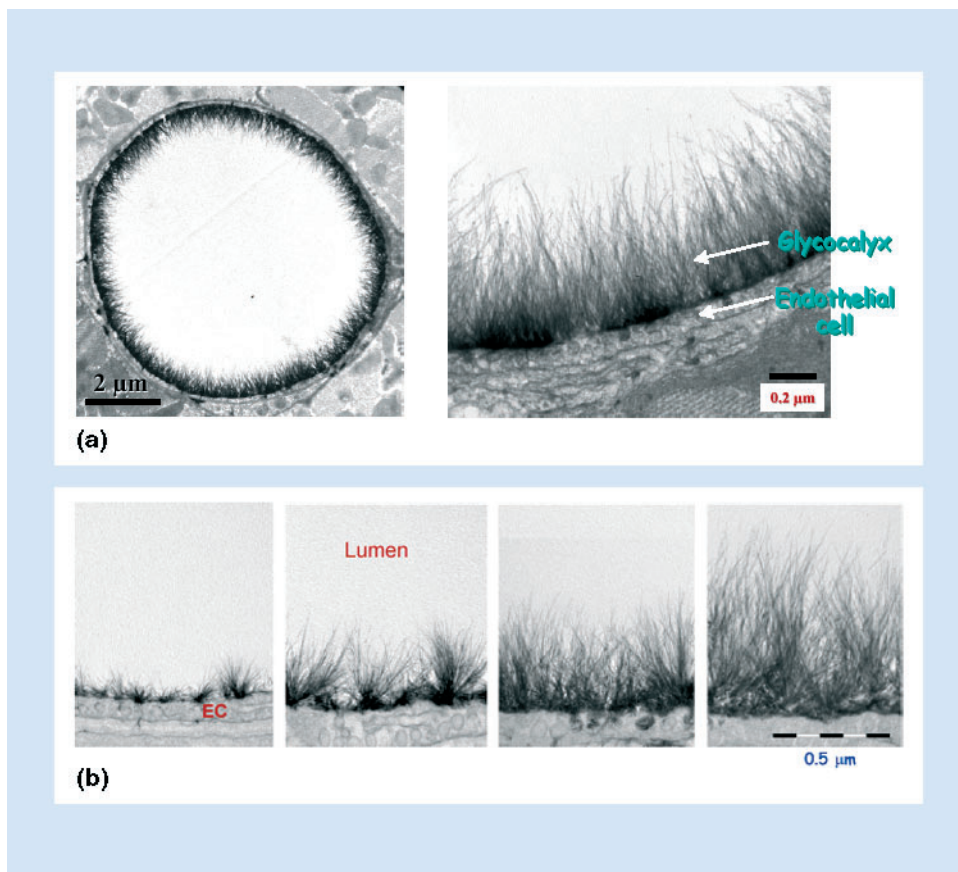


Fig. 1 (a) Electron micrographs of goat capillary glycocalyx and (b) examples of the spatial heterogeneity of glycocalyx dimensions in the vascular system (courtesy of Dr Bernard van den Berg).

vasculoprotective paradigm for larger vessels. The latter is predominantly due to the fact that glycocalyx research has traditionally focused at the microvasculature, in which atherosclerosis does not occur. However, several studies have emphasized that the relevance of the glycocalyx is not confined to smaller vessels [6, 17]. For example, van Haaren *et al.* [5] recently visualized a thick endothelial glycocalyx in larger arteries in rats. The glycocalyx in larger vessels has also been shown to decrease extravasation of LDL particles into the subendothelial space [31, 32]. Amongst others, these data imply that also in the macrovasculature the glycocalyx adds towards the vasculoprotective properties of the vessel wall.

Glycocalyx at arterial bifurcations

Although reduced levels of surface-bound sialic acids [33] and increased endothelial permeability

and susceptibility to atherosclerotic lesion formation [18] have been found to coincide with arterial branch points and curvatures, little is known about the contribution of glycocalyx perturbation to the increased vascular vulnerability of high atherogenic risk areas. Atherosclerotic lesions within the arterial tree develop at predictable vessel geometries, e.g. arterial branching and curvatures, and constraints on vessel motion by the surrounding tissues, which lead to local flow instabilities and separations. Such lesions can be detected and visualized as changes in vascular wall properties and quantified as intima-to-media ratios (IMR). Increases in IMR have been found to be associated with increased cardiovascular risk factors and atherosclerosis [34–36].

In a recent study, van den Berg *et al.* [6] hypothesized that endothelial cells, which play a central role in response to shear stress [37], express a modified surface glycocalyx at high

atherogenic risk regions and, in turn, contribute to predisposition of these arterial sites to atherosclerotic lesion formation. The endothelial glycocalyx dimension was investigated by electron microscopic observation at low- and high-risk regions of the C57Bl/6J mouse carotid artery, using the common- and internal carotid bifurcation (sinus) area as a model for arterial sites exposed to low- and high-atherogenic risk, respectively [38]. As shown in Fig. 2, it is clear that the dimension of the endothelial glycocalyx at the sinus region of the mouse internal carotid artery is significantly smaller than the glycocalyx dimension on the luminal surface of the common carotid artery. This finding is in support of the hypothesis that

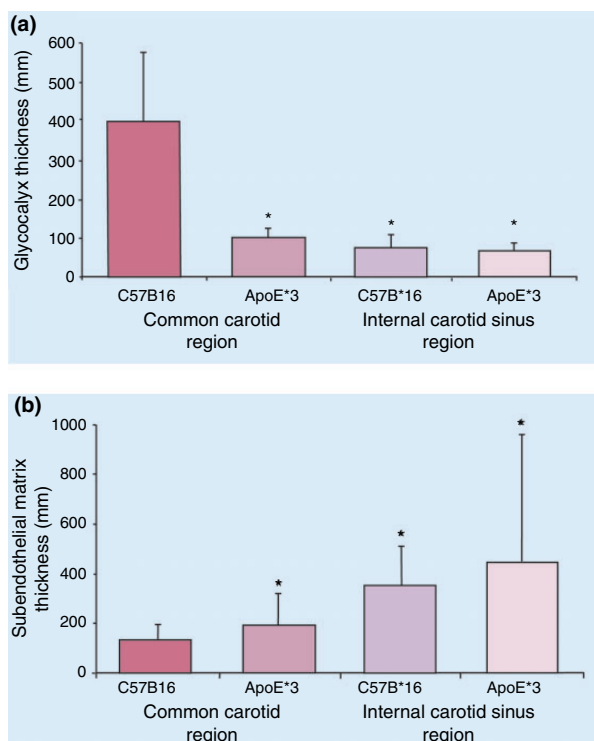


Fig. 2 (a) Glycocalyx dimension is diminished at the atheroprone sinus region of the internal carotid artery in mice compared with the atheroprotected common carotid artery. Systemic atherogenic stimulation by a hyperlipidaemic, hypercholesterolaemic diet for 6 weeks in ApoE3-Leiden mice further diminishes the dimension of the glycocalyx in the common carotid artery (from reference van den Berg *et al.* [6]). * $P < 0.05$ compared with common region of C57Bl6 mice on normal diet. (b) Greater dimensions of the subendothelial intima layer result in greater intima-to-media ratios (IMR) at vulnerable sites of the carotid arterial bifurcation with diminished glycocalyx dimensions (from reference van den Berg *et al.* [6]). * $P < 0.05$ compared with common region of C57Bl6 mice on normal diet.

perturbation of the glycocalyx contributes to the increased vascular vulnerability of regions that are at high atherogenic risk. Furthermore, this thinner glycocalyx is accompanied by greater IMR and a thicker subendothelial layer, indeed confirming that regional differences in glycocalyx dimension reflect variations in its vasculoprotective capacity.

Previous studies have demonstrated that loss of GAGs from the endothelial glycocalyx by enzyme treatment is associated by oedema formation of the subendothelial space [4], indicating that flow profile-related modulation of the glycocalyx might contribute to the earlier observed progression from a decreased endothelial barrier function into subsequent intimal oedema at vascular regions exposed to disturbed flow [39]. Whether oedema formation contributed to the increased IMR in the present study remains to be explored. However, the site-specific differences in IMR occurred in the absence of changes in the dimension of the media layer, and were predominantly because of increases in the dimension of the subendothelial space. Furthermore, no evidence was found for accumulation of blood cells or monocytes in the intima layer, indicating that the contribution of the inflammatory response was minimal at this stage.

Mechanism of glycocalyx reduction at high-risk regions

The fact that the glycocalyx dimension is significantly diminished at the sinus region compared with the glycocalyx dimension at the opposite site of the internal carotid near the flow divider as well as at the common carotid area just proximal to the carotid bifurcation, suggests that spatial differences in glycocalyx dimension are related to local variations in flow profiles. It is well known that areas of high atherogenic risk are located close to regions of disturbed flow at arterial bifurcations. Therefore, it is tempting to speculate that undisturbed flow patterns and the associated stimulation of vascular endothelium by fluid shear stress are essential to obtain optimal glycocalyx-protective properties. However, although studies have recently demonstrated that the endothelial glycocalyx indeed plays an important role in mechanotransduction of fluid shear stress, very few data are available on the relationship between fluid shear stress and glycocalyx synthesis.

Earlier studies, using sialic acid-binding lectins [33] and alcian blue [18], showed that reduced dimensions of the endothelial glycocalyx at arterial sites exposed to disturbed flow patterns associate with increases in endothelial permeability and susceptibility to atherosclerotic lesion formation. Additionally, studies by Woolf [40] and Wang *et al.* [41] revealed thicker glycocalyces at high shear regions compared with low shear regions and demonstrated that glycocalyx dimension is reduced when rabbits are fed an atherogenic diet. Steady-state glycocalyx dimension is the result of local synthesis and degradation of its constituents and it is important to know the factors that determine this balance.

Recently, Gouverneur *et al.* [42] demonstrated that exposure of cultured endothelial cells for 24 h to a shear stress of 10 dynes cm^{-2} stimulates incorporation of glucosamine-containing GAGs in the glycocalyx, which is accompanied by elevated levels of glucosamine-containing GAGs in the supernatant. These increases were confirmed by direct demonstration of increased hyaluronan concentrations in the glycocalyx and in the supernatant, as well as by a threefold increase in the incorporation of hyaluronan-binding protein in the glycocalyx. In addition to its incorporation in hyaluronan, glucosamine is also incorporated in sulphated sugars like heparan sulphate and chondroitin sulphate. In addition, Arisaka *et al.* [43] used pig aortic endothelial cells exposed to shear stress levels of 15 and 40 dynes cm^{-2} in a parallel flow chamber for periods of 3, 6, 12 and 24 h. These authors demonstrated increased synthesis of sulphated GAGs after high shear stress of 40 dynes cm^{-2} , and also a small, but significant increase at 15 dynes cm^{-2} . Similarly, Elhadj *et al.* [44] exposed bovine aortic endothelial cells for 7 days to <0.5 dynes cm^{-2} prior to increasing shear rates for 3 days to 5 and 23 dynes cm^{-2} . No significant increase in the net sulphated GAG synthesis was detected, but a shift in its size distribution was reported, indicating that modulation of specific sulphation patterns may occur despite limited effects on sulphated GAG synthesis. In summary, these experiments demonstrate that shear stress increases hyaluronan content in the endothelial glycocalyx, that shear stress exposure alters the size distribution of endothelial sulphated GAGs, and that high levels of shear stress may also increase sulphated GAG synthesis.

Glycocalyx and systemic atherogenic stimuli

In addition to the spatial differences in glycocalyx dimension at arterial bifurcations, van den Berg *et al.* [6] also reported that the glycocalyx is diminished upon systemic atherogenic challenge by a high-fat, high-cholesterol diet. Systemic perturbation of the glycocalyx by hypercholesterolaemia and/or hypertriglyceridaemia on top of pre-existing regional variations in glycocalyx-protective properties, introduced further increases in vascular vulnerability. The mechanism by which the glycocalyx is diminished in atherogenic mice remains to be elucidated, but the present finding is consistent with previous studies demonstrating rapid shedding of glycocalyx from the endothelial surface upon acute stimulation with elevated plasma levels of Ox-LDL or by acute exposure of the endothelium to inflammatory agents like thrombin or tumour necrosis factor- α [45–47]. In conclusion, both regional and risk factor-induced increases in atherogenic risk are associated by smaller glycocalyx dimensions and greater IMR. Exposure of the high-risk sinus area to an additional atherogenic challenge results in endothelial thickening and excessive swelling of the subendothelial space, in line with the proposed hypothesis that vascular sites with diminished glycocalyx are more vulnerable to pro-inflammatory and atherosclerotic sequelae.

Human glycocalyx measurements

To date, direct visualization of endothelial glycocalyx in humans has been unsuccessful, mainly due to the fact that the endothelial glycocalyx is a very delicate structure depending critically on the presence of flowing plasma [2]. As a consequence, the best way to measure the endothelial glycocalyx in humans is to compare systemic intravascular distribution volumes for glycocalyx permeable versus glycocalyx impermeable tracers. Subtracting these two volumes provides an estimate of whole body glycocalyx volume [48].

At present, Nieuwdorp *et al.* [48] tried to answer the question whether glycocalyx perturbation-mediated vascular vulnerability contributes to the accelerated rate of atherogenesis in patients with type 1 diabetes. Whereas this is at least in part the

consequence of increased prevalence of traditional cardiovascular risk factors, these cannot fully explain the propensity towards cardiovascular complications in diabetic patients [49]. Disease-specific abnormalities, such as hyperglycaemia, may also facilitate the development of vascular lesions in these patients. Thus, hyperglycaemia has been shown to induce a wide array of downstream effects, which may adversely affect the protective capacity of the vessel wall [50]. As increased degradation of proteoglycans has indeed been demonstrated in hyperglycaemic conditions [51, 52], the impact of hyperglycaemia on the glycocalyx merits special interest. Therefore, Nieuwdorp *et al.* recently set out to evaluate the impact of hyperglycaemia on the glycocalyx in healthy volunteers. Systemic glycocalyx volume was measured before and 6 h after normo-insulinaemic, hyperglycaemic clamping.

Interestingly, Nieuwdorp *et al.* demonstrated that glycocalyx constitutes a large intravascular compartment of up to 2 L in healthy volunteers (Fig. 3), which can be estimated in a reproducible fashion. More importantly, they show that hyperglycaemic clamping elicits a profound reduction in glycocalyx volume coinciding with increased circulating plasma levels of glycocalyx constituents like hyaluronan, consistent with release of glycocalyx constituents into the circulation upon hyperglycaemia. These disturbances are accompanied by impaired flow-mediated dilation as well as activation of the coagulation system. Taken in conjunction with available experimental data, the present findings imply that glycocalyx perturbation may be a novel mechanism contributing to enhanced vulnerability

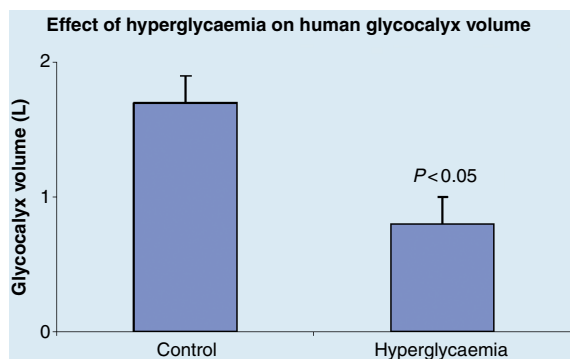


Fig. 3 Effect of 6 h acute hyperglycaemia (16 mmol l^{-1}) on systemic glycocalyx volume in healthy human volunteers (reproduced from Nieuwdorp *et al.* [48]).

of the vessel wall under hyperglycaemic conditions. Similarly, several other research groups have reported endothelial dysfunction under hyperglycaemic conditions [53, 54]. Whereas impaired NO bioavailability has predominantly been adjudicated to direct inactivation of NO by increased radical production [55, 56], the present finding provides us with an alternative option. It has been acknowledged that the glycocalyx serves as part of the endothelial mechanosensor, which translates intravascular shear stress into biochemical activation of endothelial cells [9–11, 57]. Accordingly, the release of NO by endothelial cells in response to shear stress is abolished upon enzymatic removal of GAGs from the endothelial glycocalyx [9, 10, 57]. It is tempting to speculate that loss of glycocalyx may have contributed to the impaired shear-mediated NO release during hyperglycaemia.

Summary

Currently available evidence in animal models shows that the glycocalyx exerts a wide array of anti-atherogenic effects via inhibition of coagulation and leucocyte adhesion, by contributing to the vascular permeability barrier as well as by mediating shear stress-induced NO release. In agreement with the hypothesis that glycocalyx perturbation increases endothelial vulnerability, the dimension of the endothelial glycocalyx at atherogenic lesion-prone sites is significantly smaller than its dimension on the luminal surface of the atheroprotected common carotid artery. Furthermore, focal sites with diminished glycocalyx dimension appear to be more sensitive to further provocation by systemic atherogenic stimuli. Most intriguing is the finding that relatively great systemic glycocalyx volumes in healthy volunteers are significantly reduced upon exposure to atherogenic risk factors. As yet, this finding does not prove causality of glycocalyx derangement in mediating elevated atherogenic risk and future studies need therefore to address whether restoration of the glycocalyx in itself is able to slow down or even reverse the progression of atherosclerotic disease. Nevertheless, systemic glycocalyx measurement may hold a promise as a diagnostic tool to estimate cardiovascular risk as well as to evaluate the impact of cardiovascular risk-lowering or even glycocalyx-restoring therapeutic interventions.

Conflict of interest statement

No conflict of interest was declared.

References

- Cheung BM, Launder IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. *Br J Clin Pharmacol* 2004; **57**: 640–51.
- Pries AR, Secomb TW, Gaetgens P. The endothelial surface layer. *Pflugers Arch* 2000; **440**: 653–66.
- Lipowsky HH. Microvascular rheology and hemodynamics. *Microcirculation* 2005; **12**: 5–15.
- van den Berg B, Vink H, Spaan JA. The endothelial glycocalyx protects against myocardial edema. *Circ Res* 2003; **92**: 592–4.
- van Haaren PM, van Bavel E, Vink H, Spaan JA. Localization of the permeability barrier to solutes in isolated arteries by confocal microscopy. *Am J Physiol* 2003; **285**: H2848–56.
- van den Berg BM, Spaan JAE, Rolf TM, Vink H. Atherogenic region and diet diminish glycocalyx dimension and increase intima media ratios at the murine carotid artery bifurcation. *Am J Physiol* 2006; **290**: H915–20.
- Henry CB, Duling BR. Permeation of the luminal capillary glycocalyx is determined by hyaluronan. *Am J Physiol* 1999; **277**(Pt 2): H508–14.
- Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC. Mechanotransduction and flow across the endothelial glycocalyx. *Proc Natl Acad Sci U S A* 2003; **100**: 7988–95.
- Mochizuki S, Vink H, Hiramatsu O *et al.* Role of hyaluronic acid in shear induced endothelium derived nitric oxide release. *Am J Phys* 2003; **285**: H722–6.
- Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circ Res* 2003; **93**: e136–42.
- Thi MM, Tarbell JM, Weinbaum S, Spray DC. The role of the glycocalyx in reorganization of the actin cytoskeleton under fluid shear stress: a bumper-car model. *Proc Natl Acad Sci USA* 2004; **101**: 16483–8.
- Li Q, Bolli R, Qiu Y, Tang XL, Murphree SS, French BA. Gene therapy with extracellular superoxide dismutase attenuates myocardial stunning in conscious rabbits. *Circulation* 1998; **98**: 1438–48.
- Maczewski M, Duda M, Pawlak W, Beresewicz A. Endothelial protection from reperfusion injury by ischemic preconditioning and diazoxide involves a SOD-like anti-O₂- mechanism. *J Physiol Pharmacol* 2004; **55**: 537–50.
- Libby P. Inflammation in atherosclerosis. *Nature* 2002; **420**: 868–74.
- Luft JH. Fine structure of capillary and endocapillary layer as revealed by ruthenium red. *Microcirc Symp Fed Proc* 1966; **25**: 1773–83.
- Baldwin AL, Winlove CP. Effects of perfusate composition on binding of ruthenium red and gold colloid to glycocalyx of rabbit aortic endothelium. *J Histochem Cytochem* 1984; **32**: 259–66.
- Beresewicz A, Czarnowska E, Maczewski M. Ischemic preconditioning and superoxide dismutase protect against endothelial dysfunction and endothelium glycocalyx disruption in the posts ischemic guinea-pig hearts. *Mol Cell Biochem* 1998; **186**: 87–97.
- Haldenby KA, Chappell DC, Winlove CP, Parker KH, Firth JA. Focal and regional variations in the composition of the glycocalyx of large vessel endothelium. *J Vasc Res* 1994; **31**: 2–9.
- Sims DE, Horne MM. Non-aqueous fixative preserves macromolecules on the endothelial cell surface: an in situ study. *Eur J Morphol* 1993; **32**: 59–64.
- Ward BJ, Donnelly JL. Hypoxia induced disruption of the cardiac endothelial glycocalyx: implications for capillary permeability. *Cardiovasc Res* 1993; **27**: 384–9.
- Rostgaard J, Qvortrup K. Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae. *Microvasc Res* 1997; **53**: 1–13.
- Klitzman B, Duling BR. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. *Am J Physiol* 1979; **237**: H481–90.
- Desjardins C, Duling BR. Heparinase treatment suggests a role for the endothelial cell glycocalyx in regulation of capillary hematocrit. *Am J Physiol* 1990; **258**: H647–54.
- Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res* 1996; **79**: 581–9.
- Damiano ER. The effect of the endothelial-cell glycocalyx on the motion of red blood cells through capillaries. *Microvasc Res* 1998; **55**: 77–91.
- Feng J, Weinbaum S. Lubrication theory in highly compressible porous media: the mechanics of skiing, from red cells to humans. *J Fluid Mech* 2000; **422**: 281–317.
- Secomb TW, Hsu R, Pries AR. Motion of red blood cells in a capillary with an endothelial surface layer: effect of flow velocity. *Am J Physiol* 2001; **281**: H629–36.
- Van Teeffelen JWGE, Dekker S, Fokkema DS, Siebes M, Vink H, Spaan JAE. Hyaluronidase treatment of coronary glycocalyx increases reactive hyperemia but not adenosine hyperemia in dog hearts. *Am J Physiol* 2005; **289**: H2508–13.
- Constantinescu AA, Vink H, Spaan JA. Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL. *Am J Physiol* 2001; **280**: H1051–7.
- Vink H, Constantinescu AA, Spaan JA. Oxidized lipoproteins degrade the endothelial surface layer: implications for platelet-endothelial cell adhesion. *Circulation* 2000; **101**: 1500–2.
- Adamson RH. Permeability of frog mesenteric capillaries after partial pronase digestion of the endothelial glycocalyx. *J Physiol* 1990; **428**: 1–13.
- Huxley VH, Williams DA. Role of a glycocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments. *Am J Physiol* 2000; **278**: H1177–85.
- Gorog P, Born GVR. Uneven distribution of sialic acids on the luminal surface of aortic endothelium. *Br J Exp Pathol* 1983; **64**: 418–24.
- Allen PL, Mowbray PI, Lee AJ, Fowkes FG. Relationship between carotid intima-media thickness and symptomatic and asymptomatic peripheral arterial disease: the Edinburgh Artery Study. *Stroke* 1997; **28**: 348–53.
- O'Leary DH, Polak JF, Kronmal RA *et al.* Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. *Stroke* 1992; **23**: 1752–60.
- Poli A, Tremoli E, Colombo A, Sirtori M, Pignoli P, Paoletti R. Ultrasonographic measurement of the common carotid artery wall thickness in hypercholesterolemic patients: a new model

- for the quantitation and follow-up of preclinical atherosclerosis in living human subjects. *Atherosclerosis* 1988; **70**: 253–61.
- 37 Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev* 1995; **76**: 519–60.
 - 38 Liepsch D. An introduction to biofluid mechanics – basic models and applications. *J Biomech* 2002; **35**: 415–35.
 - 39 Fry DL. Arterial intimal-medial permeability and coevolving structural responses to defined shear-stress exposures. *Am J Physiol* 2002; **283**: H2341–55.
 - 40 Woolf N. The arterial endothelium. In: Crawford ST, ed. *Pathology of Atherosclerosis*. London, England: Butterworths & Co Ltd, 1982: 25–45.
 - 41 Wang S, Okano M, Yoshida Y. Ultrastructure of endothelial cells and lipid deposition on the flow dividers of branchiocephalic and left subclavian arterial bifurcations of the rabbit aorta. *J Jpn Atheroscler Soc* 1991; **19**: 1089–100.
 - 42 Gouverneur M, Spaan JA, Pannekoek H, Fontijn RD, Vink H. Fluid shear stress stimulates incorporation of hyaluronan into the endothelial cell glycocalyx. *Am J Physiol Heart Circ Physiol* 2006; **290**: H458–62.
 - 43 Arisaka T, Mitsumata M, Kawasumi M, Tohjima T, Hirose S, Yoshida Y. Effects of shear stress on glycosaminoglycan synthesis in vascular endothelial cells. *Ann N Y Acad Sci* 1995; **748**: 543–54.
 - 44 Elhadj S, Mousa SA, Forsten-Williams K. Chronic pulsatile shear stress impacts synthesis of proteoglycans by endothelial cells: effect on platelet aggregation and coagulation. *J Cell Biochem* 1902; **86**: 239–50.
 - 45 Constantinescu AA, Vink H, Spaan JAE. Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1541–7.
 - 46 Henry CB, Duling BR. TNF- α increases entry of macromolecules into luminal endothelial cell glycocalyx. *Am J Physiol* 2000; **279**: H2815–23.
 - 47 Subramanian SV, Fitzgerald ML, Bernfield M. Regulated shedding of syndecan-1 and -4 ectodomains by thrombin and growth factor receptor activation. *J Biol Chem* 1997; **272**: 14713–20.
 - 48 Nieuwdorp M, van Haeften TW, Gouverneur MCLG *et al.* Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. *Diabetes* 2006; **55**: 480–6.
 - 49 Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339**: 229–34.
 - 50 Nathan DM, Lachin J, Cleary P *et al.* Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med* 2003; **348**: 2294–303.
 - 51 Ceriello A, Giugliano D, Dello Russo P, Passariello N, Saccomanno F, Sgambato S. Glycosaminoglycans in human diabetes. *Diabetes Metab* 1983; **9**: 32–4.
 - 52 Ikegami-Kawai M, Okuda R, Nemoto T, Inada N, Takahashi T. Enhanced activity of serum and urinary hyaluronidases in streptozotocin-induced diabetic Wistar and GK rats. *Glycobiology* 2004; **14**: 65–72.
 - 53 Krentz AJ, Boyle PJ, Macdonald LM, Schade DS. Octreotide: a long-acting inhibitor of endogenous hormone secretion for human metabolic investigations. *Metabolism* 1994; **43**: 24–31.
 - 54 Title LM, Cummings PM, Giddens K, Nassar BA. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *J Am Coll Cardiol* 2000; **36**: 2185–91.
 - 55 Timimi FK, Ting HH, Haley EA, Roddy MA, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1998; **31**: 552–7.
 - 56 Cosentino F, Luscher TF. Tetrahydrobiopterin and endothelial function. *Eur Heart J* 1998; **19** (Suppl. G): G3–8.
 - 57 Pohl U, De Wit C, Gloe T. Large arterioles in the control of blood flow: role of endothelium-dependent dilation. *Acta Physiol Scand* 2000; **168**: 505–10.
- Correspondence:* Hans Vink PhD, Department of Medical Physics, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.
(fax: 31 (0)20 6917233; e-mail: h.vink@amc.uva.nl).