

Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL

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Received 6 July 2000; accepted in final form 28 August 2000

Constantinescu, Alina A., Hans Vink, and Jos A. E. Spaan. Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL. *Am J Physiol Heart Circ Physiol* 280: H1051–H1057, 2001.—Proteoglycans and plasma proteins bound to the endothelial cell glycocalyx are essential for vascular function, but at the same time, they lower capillary tube hematocrit by reducing capillary volume available to flowing blood. Because oxidized low-density lipoproteins (oxLDL) reduce the effective thickness of the glycocalyx (Vink H, Constantinescu AA, and Spaan JAE. *Circulation* 101: 1500–1502, 2000), we designed the present study to determine whether this is caused by pathological degradation of glycocalyx constituents or increased glycocalyx deformation by elevated shear forces of flowing blood. Capillaries from the right cremaster muscle of 24 hamsters were examined by using intravital microscopy after systemic administration of normal LDL ($n = 4$), moderate oxLDL (6-h oxidation with CuSO_4 , $n = 7$), severe oxLDL (18-h oxidation, $n = 5$), and moderate oxLDL plus superoxide dismutase (SOD) and catalase ($n = 8$). Capillary tube hematocrit increased from 0.16 ± 0.03 to 0.37 ± 0.05 and from 0.15 ± 0.01 to 0.31 ± 0.03 after moderate oxLDL and severe oxLDL, respectively. These changes were paralleled by increases in red blood cell flux from 8.7 ± 1.9 to 13.8 ± 3 and from 10.7 ± 2.1 to 16.3 ± 3.2 cells/s after moderate oxLDL and severe oxLDL, respectively, in the absence of changes in anatomic capillary diameter. Red blood cell velocity, as a measure for the shear forces on the glycocalyx, was not affected by oxLDL, whereas tissue pretreatment with SOD and catalase completely abolished the effects of oxLDL on glycocalyx thickness, capillary hematocrit, and red blood cell flux. We conclude that elevation of capillary tube hematocrit by oxLDL reflects degradation of the endothelial glycocalyx by oxygen-derived free radicals.

endothelial surface layer; oxygen radicals

CAPILLARY TUBE HEMATOCRIT is defined as the instantaneous volume fraction of a capillary blood vessel filled with red blood cells (RBCs). Capillary tube hematocrit ranges from 20–50% of large vessel (systemic) hematocrit (7, 14, 15, 28) and an increasing amount of evidence relates low values of capillary tube hematocrit to the presence of the endothelial cell glycocalyx, a thick endothelial surface layer extending 0.5 μm into the capillary lumen, thereby reducing functionally per-

fused capillary volume (7, 37). Local perfusion of microvascular beds with heparinase or stimuli elevating organ blood flow (7, 15) increases capillary tube hematocrit, indicating that both enzymatic degradation and shear-dependent compression of the endothelial cell glycocalyx by RBCs may increase functionally perfused capillary volume.

The endothelial glycocalyx consists of specific proteoglycans and glycoproteins attached to the endothelial cell membrane binding a large number of plasma proteins essential for vascular function (1, 12, 30, 31). Degradation of the endothelial glycocalyx increased accumulation of plasma macromolecules into the vascular wall (10a, 36) and impaired protein binding to the luminal endothelial surface resulting in associated loss of endothelial function (13, 22). Recently, we reported (35) that clinically relevant levels of atherogenic oxidized lipoproteins (oxLDL) diminish the effective thickness of the endothelial cell glycocalyx as estimated from the distance of flowing RBCs to the luminal capillary endothelial surface. The reduction of endothelial cell-RBC (EC-RBC) distance was paralleled by increased platelet-endothelial cell adhesion and could be prevented by the administration of superoxide dismutase (SOD) and catalase, suggesting that the effective decrease in glycocalyx thickness resulted from free radical-mediated modification of glycocalyx structures rather than from increased glycocalyx compression caused by altered hemodynamic forces on the endothelial cell surface.

In the present study, we hypothesized that atherogenic levels of oxLDL increase capillary tube hematocrit as a result of glycocalyx degradation. Capillary tube hematocrit was determined as a measure for functionally perfused capillary volume, and capillary RBC velocity was measured to monitor possible changes in hemodynamic forces on the endothelial surface. Although our study was undertaken in skeletal muscle tissue, degradation of the endothelial glycocalyx and associated loss of vascular endothelial function by atherogenic stimuli, such as oxLDL, is likely to affect other tissues as well. With respect to possible glycocalyx damage by oxLDL, the myocardium may be of special interest because of the established risk that

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oxLDL imposes on the coronary vasculature in relationship to endothelial dysfunction during development of atherosclerosis. In this regard, electron microscopic studies provide evidence of coronary endothelial glycocalyx disruption in ischemia-reperfusion (2), dietary cholesterol challenge (19, 29), and hypoxia (38). In addition, consistent with reports on the limiting role of the glycocalyx for skeletal muscle capillary hematocrit, examination of histological sections of myocardial tissue indicates similar low and variable values of capillary hematocrit in the coronary circulation (34). Therefore, monitoring changes in skeletal muscle capillary tube hematocrit, as a measure for perfused capillary volume, may be the best tool available to detect dynamic changes of endothelial glycocalyx thickness reflecting altered levels of proteoglycan-bound proteins and associated vascular dysfunction in response to elevated plasma levels of atherogenic lipoproteins.

MATERIALS AND METHODS

Animal preparation. Male golden hamsters ($n = 24$; 139 \pm 7 g body wt) were anesthetized with intraperitoneal pentobarbital sodium (35 mg/ml, 70 mg/kg body wt), and the trachea was cannulated to ensure a patent airway. The left femoral vein was cannulated for continuous infusion of 0.9% saline (0.5 ml/h) containing 10 mg/ml pentobarbital sodium to replace fluid loss and to maintain anesthesia. Each hamster was placed on a Plexiglas platter, and the right cremaster muscle was prepared for visualization of the microcirculation as previously described (15). The muscle was continuously superfused at 5 ml/min with a bicarbonate-buffered physiological salt solution (in mM: 131.9 NaCl, 4.6 KCl, 2.0 CaCl₂, 1.2 MgSO₄, and 20 NaHCO₃) gas-equilibrated with 5% CO₂-95% N₂ to obtain a pH of 7.35 to 7.45. Succinylcholine (10⁻⁵ M, Sigma Chemical, St. Louis, MO) was added to the superfusion solution to reduce spontaneous skeletal muscle contractions. The cremaster muscle was maintained at 34°C by controlling the temperature of the superfusate solution, whereas esophageal temperature was maintained between 37 and 38°C with conducted heat. All procedures were performed in accordance with the institutional guidelines for animal welfare.

Intravital microscopy. Microvessels of the cremaster muscle were examined with an intravital microscope (BHM, Olympus) and a cooled, intensified charge-coupled device video camera (GenIV ICCD, Princeton Instruments). The tissue was transilluminated with a mercury lamp (100 W) equipped with a 435-nm bandpass interference filter (blue light) using an aplanat, achromatic condenser set at numerical aperture (NA) 1.2 (U-AAC, Olympus). The tissue was examined with a $\times 60$ magnification water immersion objective lens (UplanApo NA 1.2 W or LUMPlanFL NA 0.9 W, Olympus) and a telescopic tube, which yielded a final $\times 250$ magnification from the object to the camera. Images were displayed on a Philips CM 8833-II video monitor and recorded by using a SVHS videotape recorder (BR-S611E, JVC) and a time coding interface unit (SA-F911E, JVC) for further image analyses.

Lipoprotein preparation and oxidation. Human LDL (L 2139, Sigma) was dialyzed against PBS for 24 h at 4°C at pH 7.4 and the final product of this dialysis, which did not suffer further treatment, was considered normal LDL (nLDL). Incubation of lipoproteins after dialysis with 7.5 μ mol/l CuSO₄ at 37°C was performed for 6 h to obtain moderate oxLDL and for 18 h to obtain severe oxLDL. In both cases, the oxidative

reaction was stopped by addition of 0.01 mmol/l EDTA, and oxLDL was further dialyzed for 48 h in PBS + 0.01% EDTA at 4°C. The degree of LDL oxidation was determined by analyzing the content of thiobarbituric acid-reactive substances (TBARS) of the sample expressed as malonaldehyde equivalents (MDA eq) (3). TBARS values averaged 0.13 \pm 0.01 nmol MDA eq/100 μ g protein for nLDL, 0.86 \pm 0.12 nmol MDA eq/100 μ g protein for moderate oxLDL, and 2.32 \pm 0.14 nmol MDA eq/100 μ g protein for severe oxLDL. The protein concentration of each sample was determined according to Lowry et al. (21) and was taken into account when determining the lipoprotein dose to be injected systemically. Samples of nLDL and oxLDL were stored at 4°C until used.

Oxygen-derived free radicals. To test whether oxygen-derived free radicals, such as superoxide anion and hydrogen peroxide, were involved in the oxLDL effect, the hamster cremaster vessels were pretreated with SOD (EC 1.15.1.1, Sigma) and catalase (EC 1.11.1.6, Sigma). SOD and catalase were continuously infused systemically (SOD, 29 U/min; catalase, 7.5 U/min) and added to the superfusate of the cremaster muscle (SOD, 50 U/ml; catalase, 50 U/ml). In addition, SOD and catalase were administered as a systemic bolus (SOD, 250 U/0.1 ml saline; catalase, 250 U/0.1 ml saline) before lipoprotein injection.

Experimental protocols. After surgery, 45–60 min were allowed for the hamster cremaster muscle to recover and reach the physiological steady state. The experimental protocols started with selection of one capillary blood vessel per animal that allowed proper visualization of the capillary wall and RBC borders. The selected capillary was recorded on the videotape starting 10–15 min before lipoprotein administration to provide a baseline condition. Lipoproteins were administered as a bolus injection through the femoral vein in four distinct protocols with one protocol being used per animal as follows: nLDL in control experiments ($n = 4$), moderate oxLDL ($n = 7$), severe oxLDL ($n = 5$), and moderate oxLDL in the presence of SOD and catalase ($n = 8$). Each capillary was examined and recorded for up to 60–80 min after lipoprotein injection. A part of the experimental protocols described here provide data on the thickness of the endothelial surface layer, which we previously reported (35) as follows: nLDL (four experiments), moderate oxLDL (five out the seven experiments), and moderate oxLDL in the presence of SOD and catalase (eight experiments). In the present study, we performed two additional experiments in the protocol involving injection of moderate oxLDL because it was not possible to determine capillary hematocrit in all moderate oxLDL experiments previously reported (35). The lipoprotein dose injected intravenously was 0.4 mg/100 g body wt hamster, which is a dose previously reported to induce endothelial dysfunction after systemic administration to rodents (17, 20) and was reported to reduce the effective thickness of the endothelial glycocalyx (35). Taking into consideration a plasma volume of 3–5 ml/100 g body wt hamster, the plasma concentration of injected lipoproteins would be 0.08–0.13 mg/ml. This should provide a clinically relevant dose of oxLDL in accordance with the oxLDL concentration reported in coronary artery disease (1–6 mg/dl) (11).

Data analysis. Video images were digitized by using a frame grabber (DT3152, PCI Local Bus) and Image-Pro Plus version 3.0 software (Media Cybernetics, Silver Spring, PA). We used an onscreen caliper with a 1 mm/0.1 mm stage micrometer for all calibrated dimensional measurements. The anatomic capillary diameter, the width of RBCs, and the dimension of the EC-RBC gap was measured from bright-field images as described by Vink and Duling (37). The

anatomic capillary diameter was measured by positioning digital calipers at the inside of the capillary wall. The functional capillary diameter was measured by using the width of RBCs by positioning digital calipers at the RBC borders. The EC-RBC gap was calculated by subtracting the functional capillary radius from the anatomic capillary radius and was used as a measure for the dimension of the endothelial glycocalyx. Capillary tube hematocrit (H) was calculated from measurements of the anatomic capillary diameter (D_a), the flux of RBC (F), and the velocity of RBC (V) in each capillary, by using the following formula

$$H = \frac{F}{V \cdot \pi/4 \cdot D_a^2} \cdot MCV \quad (1)$$

where MCV is the mean corpuscular volume of hamster RBCs ($61 \mu\text{m}^3$).

During slow-motion video playback, RBC flux was determined by the time necessary for at least 50 RBCs to pass through a certain point chosen inside the capillary segment and was calculated as cells per second. The velocity of RBCs in the capillary was determined by measuring the length of a capillary segment and dividing it by the time required for RBCs to traverse this segment.

For each capillary, all parameters were determined by 30 measurements at every 10 min during the examination period. These measurements were averaged for each individual capillary, and the resulting values were again averaged among all capillaries from each experimental protocol, yielding the final values reported here.

Statistical analysis. Data are presented as means \pm SE for each group of experiments. Capillary hematocrit, RBC flux, velocity, and EC-RBC gap values were compared with their respective controls (preinjection values) by using a paired *t*-test (two-way) to test for significance at $P < 0.05$.

RESULTS

Capillary hematocrit was measured in vessels with diameters between 4.2 and 9.7 μm , the mean diameter being $6.4 \pm 0.3 \mu\text{m}$ ($n = 24$). In all four protocols, the anatomic diameter of the vessels remained constant during the time course of our experiments (Fig. 1).

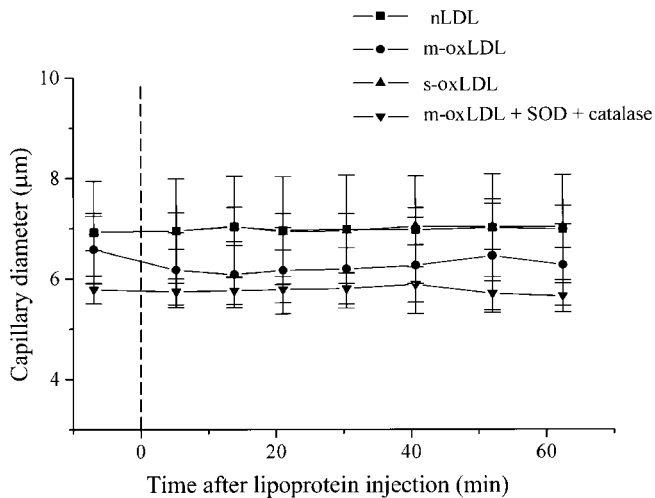


Fig. 1. Capillary anatomic diameter did not change after the administration of either normal LDL (nLDL), 6-h oxidized LDL (m-oxLDL), 18-h oxidized LDL (s-oxLDL), or m-oxLDL in the presence of superoxide dismutase (SOD) and catalase. Dashed vertical line, *time 0*.

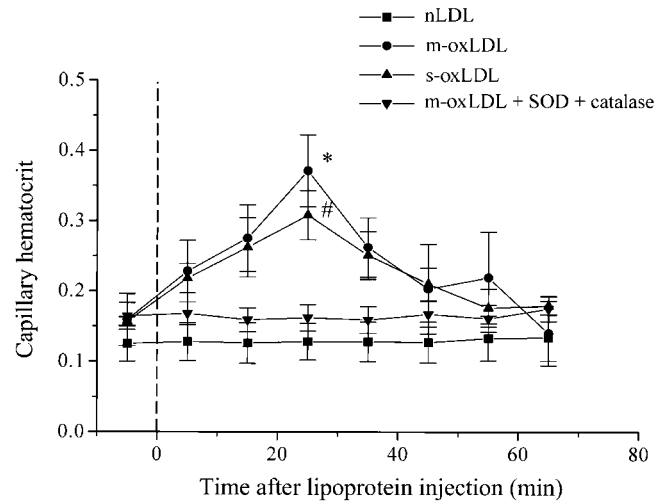


Fig. 2. Capillary tube hematocrit increased to $233 \pm 40\%$ of control after the administration of m-oxLDL ($*P < 0.01$) and to $196 \pm 25\%$ of control after the administration of s-oxLDL ($\#P < 0.01$), but remained constant in response to nLDL or m-oxLDL in the presence of SOD and catalase.

Capillary hematocrit increased transiently, reaching a maximum value in the interval (20–25 min) after injection of moderate oxLDL and severe oxLDL, and returned to the baseline value ~ 60 min after injection (Fig. 2). In control experiments, capillary hematocrit remained constant after nLDL (baseline, 0.13 ± 0.02 ; at 20 min, 0.12 ± 0.02 ; $P = 0.82$). The administration of moderate oxLDL increased capillary hematocrit to $233 \pm 40\%$ of the baseline, i.e., from 0.16 ± 0.03 before injection to 0.37 ± 0.05 ($P < 0.01$). Similarly, the administration of severe oxLDL increased capillary hematocrit to $196 \pm 25\%$ of baseline, from 0.15 ± 0.01 to 0.31 ± 0.03 ($P < 0.01$). Combined administration of moderate oxLDL with SOD and catalase to prevent damage by oxygen-derived free radicals inhibited the effect of oxLDL on capillary hematocrit (*time 0*, 0.16 ± 0.02 ; at 25 min, 0.16 ± 0.02 ; $P = 0.94$).

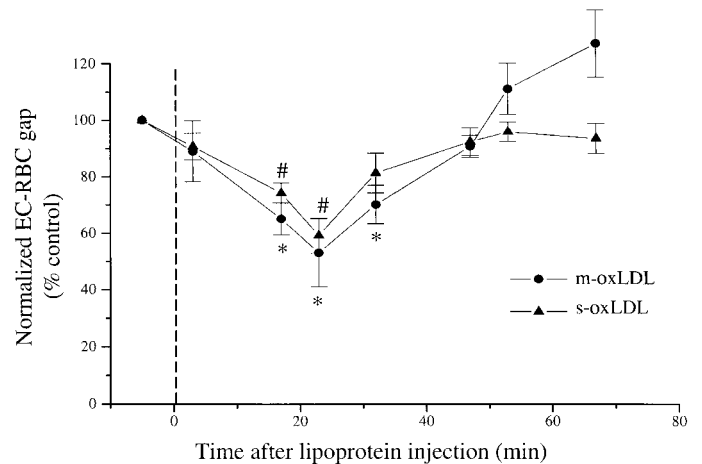


Fig. 3. The dimension of endothelial cell-red blood cell (EC-RBC) gap as a measure of the endothelial glycocalyx decreased to $53 \pm 12\%$ of control after the administration of m-oxLDL ($*P < 0.05$) and to $59 \pm 6\%$ of control after the administration of s-oxLDL ($\#P < 0.01$).

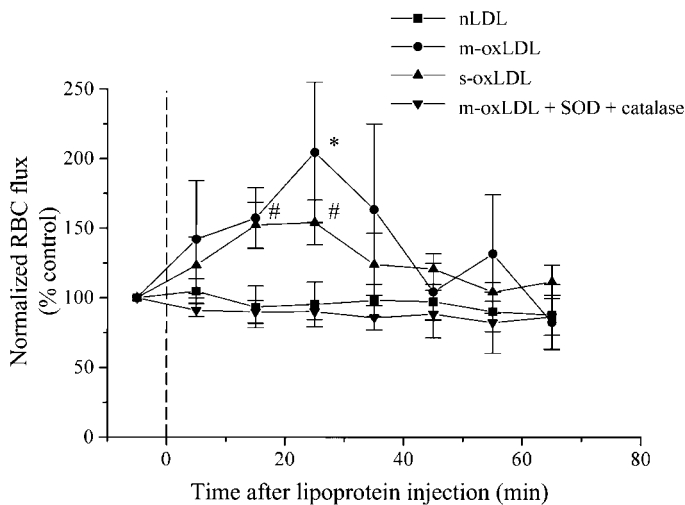


Fig. 4. RBC flux increased to $204 \pm 50\%$ of control after m-oxLDL ($*P < 0.05$) and to $154 \pm 16\%$ of control after s-oxLDL ($\#P < 0.05$) but did not change after nLDL or m-oxLDL in the presence of SOD and catalase.

The dimension of the endothelial glycocalyx was estimated by measurement of the EC-RBC gap (Fig. 3). In a previous study (35), we reported endothelial glycocalyx decreased to half of the control value after moderate oxLDL, but remained constant after nLDL and moderate oxLDL in the presence of SOD and catalase. In the experiments determining capillary tube hematocrit, the administration of moderate oxLDL reduced the EC-RBC gap to $53 \pm 12\%$ of the control value, i.e., from 0.65 ± 0.13 to $0.33 \pm 0.09 \mu\text{m}$ ($P < 0.05$), and severe oxLDL consistently decreased the EC-RBC gap to $59 \pm 6\%$ of the control value 20–25 min after administration, i.e., from 0.55 ± 0.02 to $0.32 \pm 0.03 \mu\text{m}$ ($P < 0.01$).

To determine whether changes in capillary hematocrit are associated with changes in RBC flux in capillaries (Fig. 4) or with changes in RBC velocity (Fig. 5), we determined the evolution of these parameters after lipoprotein injection. RBC flux, as a measure for capillary RBC perfusion, increased after moderate oxLDL from 8.7 ± 1.9 to 13.8 ± 3 cells/s ($P < 0.05$) and after severe oxLDL from 10.7 ± 2.1 to 16.3 ± 3.2 cells/s ($P < 0.05$). No significant changes were found after nLDL (*time 0*, 12.5 ± 4 cells/s; at 20 min, 13.1 ± 6.6 cells/s, $P = 0.81$) or after the administration of moderate oxLDL in the presence of SOD and catalase (*time 0*, 7.2 ± 0.9 cells/s; at 25 min, 6.4 ± 0.8 cells/s; $P = 0.07$). RBC velocity did not change significantly after moderate oxLDL (*time 0*, $122 \pm 46 \mu\text{m/s}$; at 23 min $75 \pm 12 \mu\text{m/s}$; $P = 0.34$) nor did it change significantly after severe oxLDL (*time 0*, 112 ± 23 to $97 \pm 32 \mu\text{m/s}$; $P = 0.31$). Similarly, no changes in RBC velocity were found after nLDL in the control experiments (*time 0*, $172 \pm 44 \mu\text{m/s}$; at 20 min, $169 \pm 64 \mu\text{m/s}$; $P = 0.89$) or after moderate oxLDL in the presence of SOD and catalase (*time 0*, $107 \pm 14 \mu\text{m/s}$; at 25 min, $97 \pm 13 \mu\text{m/s}$; $P = 0.39$).

DISCUSSION

We determined the effect of oxLDL on capillary tube hematocrit and capillary RBC velocity to test the hy-

pothesis that oxLDL decreases the effective glycocalyx dimension by removal of endothelial surface structures independent of shear-induced glycocalyx deformation (35). Capillary tube hematocrit increased in parallel with a transient decrease in glycocalyx dimension after the administration of oxLDL in the absence of significant changes in capillary RBC velocity. These findings support the hypothesis that oxLDL increases capillary volume accessible to RBCs by removal of proteoglycans or adsorbed proteins from the endothelial surface.

Determinants of capillary tube hematocrit. Microscopic observation of capillary beds *in vivo* shows that capillary tube hematocrit is much lower than systemic hematocrit (7, 14, 15, 28). Possible phenomena accounting for the observed difference include microvascular network events, such as phase separation of RBCs and plasma at upstream bifurcations or intercapillary heterogeneity of blood flow, and intracapillary events, such as different velocities for RBCs and plasma inside capillaries (Fahraeus effect) (9) or retardation of a plasma layer close to the capillary wall (15). More recent data implicate the endothelial cell glycocalyx as a wall structure with a considerable thickness in capillaries (37) that may retard intracapillary plasma flow and reduce functionally perfused capillary volume and act, therefore, as an important determinant of capillary tube hematocrit (7, 37).

Although capillary filling with RBCs is low under resting conditions, capillary tube hematocrit may increase with adenosine and tissue metabolic activity and may decrease by high oxygen exposure or reduced flow velocity (7, 15). In addition to the flow-dependent variations of capillary tube hematocrit, Desjardins and Duling (7) reported that heparinase treatment of the capillary endothelial glycocalyx increased capillary hematocrit two- to threefold independently of RBC velocity. They suggested that capillary hematocrit increases as a result of removal of heparan sulfate proteoglycans from the capillary wall. Furthermore, Pries et al. (26) reported that heparinase treatment of microvascular

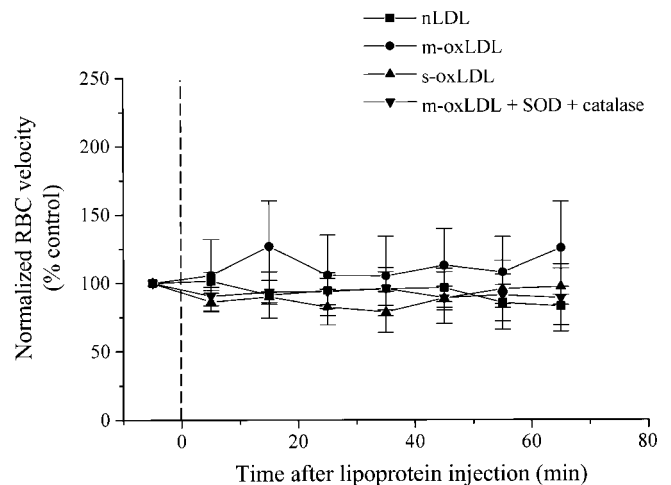


Fig. 5. RBC velocity remained constant in response to either nLDL, m-oxLDL, s-oxLDL, or m-oxLDL in the presence of SOD and catalase.

networks decreases flow resistance by 15–20%, indicating that the endothelial surface glycocalyx contributes significantly to microvascular resistance.

In our present study, capillary anatomic diameter did not change after exposure to oxLDL and neither did RBC velocity. Hence, the increased capillary hematocrit and RBC flux indicates an increased functionally perfused capillary volume caused by the degradation of the endothelial surface layer by oxLDL rather than modified capillary RBC hemodynamics with consequences on shear-dependent compression of the endothelial glycocalyx.

However, oxLDL may also affect microvascular tone, because it is reported that oxLDL decreases nitric oxide (NO) production of endothelial cells (33) and impairs NO-mediated dilation of isolated coronary arterioles to flow and adenosine (10). This raises the question of whether the oxLDL effect on capillary hematocrit observed in the present study might be caused by a direct effect of oxLDL on the vascular tone of terminal arterioles proximal to the capillary bed. In this way, inhibition of NO-mediated dilation could also be associated with an altered level of capillary hematocrit. Unpublished data from our laboratory show that inhibition of NO synthesis with L-arginine analog, N^G -nitro-L-arginine methyl ester (L-NAME), does not affect capillary hematocrit at concentrations that decrease arteriolar diameter. Furthermore, arteriolar vasoconstriction to various agents actually lowers capillary tube hematocrit (7, 15), indicating that a possible effect of oxLDL on microvascular tone because of impaired NO activity is unlikely to cause the increase in capillary hematocrit observed in the present study.

Nevertheless, an effect of oxLDL on NO bioactivity may contribute to the degradation of the endothelial glycocalyx by further disturbing the balance between oxygen radical production and NO availability at the endothelial surface. This would be consistent with disruption of the endothelial glycocalyx as observed by electron microscopy in posts ischemic guinea pig hearts involving production of oxygen radicals, whereas perfusion with L-NAME in aerobic medium does not change glycocalyx appearance (2).

Degradation of the endothelial glycocalyx in vivo. The mechanism responsible for the degradation of the endothelial glycocalyx in vivo remains to be elucidated. The protective effect of the administration of SOD and catalase indicates that oxygen-derived free radicals mediated glycocalyx degradation by oxLDL in the present study and during exposure to light-dye (37). We aimed to differentiate in the present study between the effects of moderate and severe oxLDL molecules. We found that 18-h oxLDL, with a high TBARS content, did not affect the endothelial glycocalyx differently from 6-h oxLDL. This suggests that early-stage modification of the LDL molecule, i.e., depletion of antioxidants and peroxidation of lipids (23), may be sufficient to induce disruption of the endothelial glycocalyx by upsetting the balance between prooxidative and antioxidative stresses at the endothelial surface, because SOD and catalase prevented the oxLDL effect.

Whether oxygen-derived free radicals cleave proteoglycans from the endothelial membrane or impair adsorption of plasma proteins to glycosaminoglycans needs to be further investigated. Previous studies using electron microscopy report hypoxia induced disruption of endothelial proteoglycans (6, 38) and this effect can be prevented by the administration of oxygen radical scavengers such as SOD (2). Furthermore, oxLDL treatment is able to disrupt heparan sulfate proteoglycans of the subendothelial matrix (24). However, the time courses of degradation and reconstitution of the endothelial glycocalyx reported here for oxLDL are too short to involve replacement of degraded proteoglycans unless preformed proteoglycans stored intracellularly are expressed on the endothelial surface. Therefore, it is more likely that impaired adsorption of plasma proteins to the glycocalyx may have contributed to the reduction of surface-layer dimension in response to oxLDL. Consistent with the time course for the recovery of the endothelial glycocalyx reported in the present study, considerable re-adsorption of plasma proteins to the glycocalyx after plasma substitution protocols occurs within 15 min (30).

oxLDL effect. oxLDL increases microvascular permeability (8, 27), albumin leakage (20), and leukocyte-endothelial cell adhesion (17, 16, 20) and, therefore, may trigger development of endothelial dysfunction in atherosclerosis. Although defined by morphological lesions of large vessels, atherosclerosis induces pathophysiological changes appearing to extend also into the microcirculation (5, 18). In this respect, our study investigated the effect of “atherogenic” concentrations of oxLDL on capillary hematocrit and capillary RBC perfusion and addressed the oxLDL-induced microvascular dysfunction, because we could not find an effect of oxLDL on systemic hematocrit values (unpublished data).

The transient effect of oxLDL on capillary hematocrit may be accounted for by a rapid clearance of oxLDL from systemic circulation by hepatic uptake. OxLDL, when injected systemically, is cleared much faster from the circulation than nLDL, inasmuch as 60–80% of ^{125}I -labeled oxLDL is taken up by the liver in the first 10 min after injection in rats in parallel with the decay in oxLDL serum concentration (32).

Effect of increased capillary hematocrit for tissue oxygen supply. Microvascular hematocrit participates in determination of tissue oxygen supply together with capillary blood flow and capillary density (28). Capillary hematocrit may have a major role particularly in nonsteady-state conditions, when the intracapillary transit time of RBCs may be an important variable for tissue oxygen supply (15).

In our experiments, capillary hematocrit and RBC flux increased in response to oxLDL by twofold, whereas RBC velocity remained constant. Because oxygen is transported in RBCs, it is suitable to reason that capillary oxygen content will increase proportionally with the oxLDL-induced increase in RBC flux. However, it is difficult to assess whether, and to what extent, the increased capillary oxygen content will in-

crease oxygen transport to the tissue or will increase oxygen radical formation at the capillary endothelial surface. However, because capillary hematocrit and flux doubled in response to oxLDL, it is possible that the high oxygen concentration may act as an additional source of oxygen-derived free radicals both inside the tissue and at the capillary endothelial surface. This may further stimulate production of inflammatory mediators and may further affect the endothelial glycocalyx. Therefore, although the effect of oxLDL was transient in the present study, presumably caused by oxLDL metabolic degradation, a continuous production of oxygen radicals, deriving from a sustained elevation of capillary hematocrit and oxygen content, may be expected in a chronic situation.

Implications for the coronary circulation. We have shown in the present study that elevated capillary tube hematocrit induced by oxLDL implies degradation of the endothelial glycocalyx and, in particular, considering the rapid time constant of this process, an altered adsorption of plasma proteins to the endothelial surface. Adsorption of plasma proteins to the cell surface proteoglycans and glycoproteins is intimately related to endothelial function (25). For example, albumin, fibrinogen, and orosomucoid adsorbed to the endothelial surface influence vascular wall permeability for solutes and macromolecules (12, 30, 31). Furthermore, lipoprotein lipase bound to endothelial cell heparan sulfate proteoglycans actively regulates lipoprotein metabolism, whereas proteoglycan-bound antithrombin III modulates the level of coagulation near the endothelial surface. Because all of these processes are affected during atherogenesis, altered adsorption of proteins to the endothelial cell surface is likely to have a major impact, especially on the coronary circulation.

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