Endothelial glycocalyx thickness and platelet-vessel wall interactions during atherogenesis

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Summary
The endothelial glycocalyx (EG), the luminal cover of endothelial cells, is considered to be atheroprotective. During atherogenesis, platelets adhere to the vessel wall, possibly triggered by simultaneous EG modulation. It was the objective of this study to investigate both EG thickness and platelet-vessel wall interactions during atherogenesis in the same experimental model. Intravital fluorescence microscopy was used to study platelet-vessel wall interactions in vivo in common carotid arteries and bifurcations of C57bl6/J (B6) and apolipoprotein E knock-out (ApoE−/−) mice (age 7 – 31 weeks). At the same locations, EG thickness was determined ex vivo using two-photon laser scanning microscopy. In ApoE−/− bifurcations the overall median level of adhesion was 48 platelets/mm2 (interquartile range: 16 – 80), which was significantly higher than in B6 bifurcations (0 (0 – 16), p = 0.001). This difference appeared to result from a significant age-dependent increase in ApoE−/− mice, while no such change was observed in B6 mice. At the same time, the EG in ApoE−/− bifurcations was significantly thinner than in B6 bifurcations (2.2 vs. 2.5 μm, respectively; p < 0.05). This resulted from the fact that in B6 bifurcations EG thickness increased with age (from 2.4 μm in young mice to 3.0 μm in aged ones), while in bifurcations of ApoE−/− mice this growth appeared to be absent (2.2 μm at all ages). During atherogenesis, platelet adhesion to the wall of the carotid artery bifurcation increases significantly. At the same location, EG growth with age is hampered. Therefore, glycocalyx-reinforcing strategies could possibly ameliorate atherosclerosis.

Keywords
Atherogenesis, carotid artery, endothelial glycocalyx, platelets, mice

Introduction
The endothelial glycocalyx (EG) is the carbohydrate-rich luminal lining of endothelial cells, that functions as barrier between blood and endothelium (1). The EG is proposed to have vasculoprotective properties, based on its capacity to prevent blood cell-vessel wall interactions, to reduce oxidative stress via incorporated enzymes, and to contribute to the vascular permeability barrier (1–4). Furthermore, EG disruption has been associated with vascular disease or endothelial dysfunction, such as occurs in diabetes (5) or ischaemia/reperfusion (6). The role of the EG in atherogenesis (i.e. the development of atherosclerotic lesions in the vessel wall) is not well understood. In mouse carotid arteries, atherogenic regions are associated with a reduction of EG thickness (7) and loss of vasculoprotective properties of the endothelium, as intimal low-density lipoprotein (LDL) accumulation is increased in areas with a thinner EG (8).

Atherosclerosis is an inflammatory process, marked by the formation of lesions in the vessel wall at predestination sites under conditions of high LDL concentrations and disturbed flow patterns (9,10). Platelets play a pivotal role during early stages of atherogenesis. In apolipoprotein E knock-out (ApoE−/−) mice fed a high-fat diet, an increasing number of platelets was observed to interact with the vessel wall of the carotid artery bifurcation, prior to the occurrence of leukocyte adhesion and lesion formation. Chronic inhibition of platelet glycoprotein Ibα prevented the formation of atherosclerotic plaques in this model (11). In addition, activated platelets have been shown to interact with the endothelium at lesion-prone sites in ApoE−/− mice, exacerbating atherogenesis via delivery of various chemokines, such as CCL5 (RANTES) and CXCL4 (platelet factor 4) (12–17).

The initial trigger for platelet adhesion and subsequent chemokine release in the course of atherogenesis has not yet been identified. From literature, a number of findings indicate that the EG might contribute to this process: 1) Chronic inhibition of hyaluronan synthesis (an important component of the EG) accelerated atherosclerotic plaque formation in ApoE−/− mice, and led to a pro-thrombotic state not caused by increased platelet activation. EG di-

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dimensions were reduced in these mice (18). 2) Oxidised LDL-mediated degradation of the EG has been shown to result in an increase of platelet-endothelium interactions in hamster cremaster capillaries (19). 3) In hyperlipidaemic mice the dimensions of the EG are reduced, especially at atherogenesis prone vascular sites (7, 8). However, no study has yet determined platelet adhesion as well as EG dimensions at an atherogenic arterial bifurcation in the same experimental model.

The aim of the present study was to assess platelet-vessel wall interactions and EG dimensions during atherogenesis in the carotid bifurcation (lesion-prone site) as compared to the common carotid artery (lesion-protected site) in ApoE−/− mice; C57bl6/J mice were used as wild-type controls. To this purpose, we combined intravital fluorescence microscopy (IFM) to quantify platelet-vessel wall interactions (20, 21), with our recently developed ex vivo method of EG visualisation and quantification in carotid arteries of mice using two-photon laser scanning microscopy (TPLSM) (22). We hypothesised that a reduction of EG thickness and an increase in platelet-vessel wall interactions during atherogenesis at lesion-prone sites occur simultaneously, which would suggest a causal relation between both.

Materials and methods

All experiments conducted were in line with institutional guidelines and approved by the local ethics committee on the use of laboratory animals. Studies were performed on male C57bl6/J (B6; n = 56) and C57bl6/J ApoE−/− mice (ApoE−/−; n = 53; Charles River, Maastricht, The Netherlands) aged 7–31 weeks. Mice were fed a normal chow to prevent accelerated atherogenesis, enabling detailed studies of changes in platelet behaviour and EG structure as a function of time and vessel location.

Surgery

To anesthetise the mice, a mixture of 75 mg/kg ketamin (Nimatek, Euvrot, Cuijk, The Netherlands) and 15 mg/kg xylazin (Xylalin, CEVA SANTE Animale BV, Naaldwijk, The Netherlands) was prepared in saline, and injected subcutaneously in a volume of 3 ml/kg. To maintain anaesthesia smaller doses of 0.88 ml/kg were administered every 30 minutes (min). Body temperature was monitored and maintained at 37ºC using a heating stage (TH60-SMZ, Linkam Scientific Instruments, Tadworth, UK).

The left jugular vein was canulated (PE 10, Sims Portex, London, UK) for administration of fluorescent dyes. The right carotid artery was exposed up to the bifurcation and a small piece of black plastic was placed underneath the distal part of the common carotid artery and the bifurcation to reduce background noise during fluorescence microscopy.

Intravital microscopy (IFM)

IFM was used to visualise spontaneous platelet-vessel wall interactions in the right common carotid artery as well as in the carotid bifurcation. The left carotid was used in case the right carotid bifurcation could not be visualised due to anatomical variation. Circulating blood platelets were labelled fluorescently by intravenous administration of 0.03 ml acridin red solution (AR, 2.0 mg/ml in saline with 5% ethanol, Chroma-Gesellschaft Schmid GmbH, Germany). The intravital microscope (Leitz, Wetzlar, Germany) was equipped with an objective for overview recordings (UO 6.5x, numerical aperture 0.18), a water immersion objective (SW 25x, numerical aperture 0.60), a 1.25x ploemopak for interchangeable filter sets, and a 100 W mercury lamp. To ensure similar light exposure in each experiment, the yield of the mercury lamp was measured every experiment.

To assess the diameter of the common carotid artery, incident illumination brightfield microscopy recordings were made with the UO 6.5x lens and a POL-cube (polariser, 50% mirror, and a crossed analyser in the imaging pathway) to cancel direct reflections (23). To visualise spontaneously interacting fluorescently labelled platelets, a N2–1 filter set (excitation 515–560 nm; dichroic 580 nm; barrier 580 nm longpass) was applied in combination with the SW 25x water dipping objective. Fluorescence microscopy was applied on the common carotid artery and subsequently on the bifurcation just upstream of the flow divider region, with a distance of at least 500 μm between the two sites (Fig. 1a). Due to movement of the artery and scattering of light, stable images could only be obtained from the upper segment of the exposed vessel (Fig. 1b). The imaged area (approximately 250 x 250 μm²) was captured at 30 frames per second into 1,000 x 1,000 pixels for 2.5 min per site using an EM-CCD camera (9100–2, Hamamatsu Photonics, Herrsching am Ammersee, Germany).

Plasma analysis

After intravital recordings, mice were sacrificed. Blood was collected in 0.1 ml 0.129 M citrate through puncture of the right ventricle. Plasma was isolated (13,000 rpm for 5 min at 4ºC) and stored at –80ºC for later analysis. Plasma levels of high-density lipoprotein (HDL), triglycerides, and total cholesterol were assessed using a Beckman/Couler Synchron LX20 plasma analyser. LDL concentration was calculated using the Friedewald formula.

Isolation and mounting of carotid arteries

For TPLSM imaging of the EG, carotid arteries were processed as previously described (22, 24, 25). In short, both carotid arteries including the bifurcation were carefully dissected, excised, and mounted on two glass micropipettes in a homebuilt perfusion chamber (IDEE, Maastricht, The Netherlands) (24). Fluorescent
labelling of vascular cell nuclei and the EG was achieved by perfusing the artery with 500 μl phosphate buffered saline (PBS, pH 7.4) containing 5 μM SYTO41 (Molecular Probes, Leiden, The Netherlands) and 2.6 μM fluorescein isothiocyanate-labelled wheat germ agglutinin (WGA-FITC; Sigma-Aldrich, Zwijndrecht, The Netherlands) at a rate of 1 ml/hour for 30 min, which results in an estimated low wall shear stress of 0.02 Pa. WGA-FITC binds sialic acids and also glucosamine-based glycosaminoglycans such as heparan sulfate and hyaluronan, providing general labelling of the EG (22).

In carotids from six ApoE-/- and two B6 mice, phycoerythrin-conjugated rat anti-mouse CD11b antibody (anti-CD11b-PE; final concentration 8 μg/ml; BD Biosciences, Alphen aan den Rijn, the Netherlands) was added to the labelling mixture as a marker for inflammatory cell migration to the subendothelium. After labelling, a transmural pressure of 40–80 mmHg was applied using a modified sphygmomanometer (Big Ben, Riester, Jungingen, Germany). Ex vivo vessel processing and labelling was performed at room temperature.

Figure 1: Imaging locations. Schematic field of view and scanning locations in longitudinal (a) and transversal (b) direction in the surgically exposed (IFM) or mounted (TPLSM) carotid artery. Recordings were made in the common carotid artery and in the carotid bifurcation. The distance between these sites was at least 500 μm. IFM was performed with a field of view of about 250 x 250 μm², while that of TPLSM images was about 200 x 200 μm². Both imaging modalities were applied to the top of the vessel, as depicted by the black rectangle in b). c) Optical section obtained using two-photon laser scanning microscopy ex vivo in the right carotid artery bifurcation of an 18-week-old ApoE-/- mouse labelled with SYTO41 (blue, cell nuclei), WGA-FITC (green, endothelial glycocalyx) and anti-CD11b-PE (red, CD11b-positive cells). CD11b-positive cells (arrowheads) were found under the endothelium. The nuclear shape hints at their differentiation; white arrowhead: polymorphonuclear granulocyte; yellow arrowhead: monocyte. Note the WGA labelling (green) of the membrane of these cells as well. Bar represents 10 μm. d) Using IFM, both adhering (circles) and saltating (arrowheads) platelets could be observed in the carotid bifurcation of a 25-week-old ApoE-/- mouse. Saltating platelets only interacted for a short period of time, while the adhering platelets stayed at one site for more than 20 seconds. e) The same vessel 2.7 seconds later. Both saltating platelets (arrowheads in d) are now gone, whereas the adhering ones are still present. Bars represent 50 μm. Recordings were made at 30 frames per second. Numbers indicate the time (in ms) from the start of the recording.

The perfusion chamber was positioned on a Nikon E600FN microscope (Nikon, Tokyo, Japan), coupled to a standard BioRad 2100 MP multiphoton system (Bio-Rad, Hemel Hempstead, UK). A 120 ± 20-fs-pulsed Ti:Sapphire laser (Spectra Physics Tsunami, Mountain View, CA, USA) tuned and mode-locked at 800 nm was used as excitation source. A 60x water dipping objective (Nikon; numerical aperture 1.0, working distance 2 mm) was used and the resulting maximum field of view was approximately 200 x 200 μm². If necessary, optical zoom could be applied to enhance cellular details (e.g. presence of CD11b-positive cells, Fig. 1c). The fluorescent signals from SYTO41, WGA-FITC and anti-CD11b-PE were detected by three photo multiplier tubes (PMTs); PMT1 (pseudo-colour blue) received the SYTO41 signal at 420–470 nm; PMT2 (pseudo-colour green) received the WGA-FITC signal at 510–550 nm; PMT3 (pseudo-colour red) received the anti-CD11b-PE signal at 570–640 nm. Single images (512 x 512 pixels) were recorded in the xy-plane and subsequently collected at successive depths (z-stack; distance between the xy-planes was 0.45 or 0.6 μm).
TPLSM and IFM recordings were made at approximately the same locations (common carotid artery and carotid bifurcation; Fig. 1a and b).

Data processing

IFM video analysis was performed offline with the Wasabi software package (version 1.5, Hamamatsu Photonics), allowing quantification of vessel diameter and frame-by-frame monitoring of platelet-vessel wall interactions. Two categories of the latter were identified: saltating and adhering platelets. Platelets that attached to the vessel wall at a specific site for at least 90 ms (4 successive images) and subsequently detached were defined as saltating and expressed as number of saltating platelets/mm²/min. Platelets that adhered to the same spot of the vessel wall for longer than 20 seconds were defined as adhering. Numbers are expressed as number of adhering platelets/mm². For examples of each category, see Figures 1D and E. Saltating platelets were found throughout each recording, while most adhering platelets were usually seen from the beginning onwards. Therefore, numbers of adhering platelets are expressed without time-scale. Heart rates were estimated from repetitive vessel wall movements in the video stream.

During IFM recording, almost no leukocytes were found interacting with the vessel wall in either strain or age group. In those few occurrences, leukocytes could be discriminated from platelets by their size and fluorescence intensity.

Data from IFM experiments were discarded when arterial movement impaired successful imaging of platelet-vessel wall interactions, when signal-to-noise ratio (SNR) was too low, or when animals died preliminarily. The final number of IFM experiments (common/bifurcation) was for B6: 33 / 24, and for ApoE⁻/⁻: 29 / 26.

TPLSM data were analysed with Imagepro software package (version 6.1, Media Cybernetics, Silver Spring, MD, USA) including the 3D-reconstructor set (version 5.1). For image computations, Matlab software (version 7.01, The MathWorks Inc., Natick, MA, USA) was used. Using a recently described image analysis protocol, EG thickness was calculated per scanned vessel segment (22).

TPLSM data were discarded when adequate mounting of the carotid artery including the bifurcation was not feasible, when

Figure 2: Platelet adhesion and EG thickness in the carotid artery of B6 and ApoE⁻/⁻ mice. Top panels: Number of adhering platelets (left) and EG thickness (right) in the carotid bifurcation of B6 and ApoE⁻/⁻ mice. Lower panels: same parameters in the common carotid artery of both strains. Data are presented as box and whisker plots showing the 10th, 25th, 50th, 75th and 90th percentiles. Outliers are not depicted. * p ≤ 0.05; *** p ≤ 0.001; NS: not significant.
WGA-labelling was incomplete, and/or when SNR was too low (22). The final number of TPLSM experiments (common / bifurcation) was for B6: 21 / 13, and for ApoE-/-: 25 / 12. The surface area labelled with WGA-FITC per vessel segment was comparable between these groups (typically >90%).

Statistical analysis

Statistical analyses were performed using the SPSS software package (version 15.0, SPSS Inc., Chicago, IL, USA). Data are presented as median (interquartile range) unless otherwise specified. Age and/or strain groups were compared using the non-parametric Mann-Whitney test. Non-parametric testing was used either because of group sizes (TPLSM data) or for reasons of skewed data distribution (IFM data). The level of significance was set at 0.05.

Results

Influence of strain and vessel site on platelet adhesion and EG thickness

The average number of adhering platelets in the carotid bifurcation of all ApoE-/- mice together was significantly higher than in all B6 bifurcations (median: 48 (interquartile range: 16 – 80) platelets/mm² vs. 0 (0 – 16), p = 0.001; Fig. 2, upper left panel). At the same time, EG thickness in the carotid bifurcation of ApoE-/- mice was significantly lower (2.2 (2.1 – 2.4) μm in ApoE-/- vs. 2.5 (2.3 – 3.0) in B6; p < 0.05; Fig. 2, upper right panel).

No strain difference was observed in the number of adhering platelets in the common carotid artery (ApoE-/- 0 (0 – 32) platelets/mm² vs. B6 0 (0 – 16); Fig. 2, lower left panel). In parallel, EG thickness in the common carotid was comparable between both strains (1.9 (1.7 – 2.3) μm in ApoE-/- vs. 2.2 (1.8 – 2.5) in B6; Fig. 2, lower right panel). In a subgroup of experiments, both IFM and TPLSM data were available from the same subjects. In these few
cases (ApoE-/-: n = 5; B6: n = 4), an inverse relation between EG thickness and the number of adhering platelets was observed (not shown).

The number of salting platelets was comparable for both strains (common: B6: 45 (10 – 147) platelets/mm²/min, ApoE-/-: 96 (29 – 176); bifurcation: B6: 115 (32 – 186), ApoE-/-: 83 (0 – 320); not shown).

Influence of age

As presented in the previous section, a significantly higher number of adhering platelets coincides with lower EG thickness in the bifurcation of ApoE-/- mice as compared to wild-type controls. However, both IFM and TPLSM data show large variation (Fig. 2). In order to explain (part of) this variation, the effect of age was considered (Fig. 3). In ApoE-/- mice, the number of adhering platelets in the carotid bifurcation increased with age (solid line in the top right panel of Fig. 3); Spearman’s rho 0.497, p = 0.01). This resulted in a significant difference between young (7 – 18 weeks) and aged (19 – 31 weeks) ApoE-/- mice (24 (0 – 52) platelets/mm² vs. 64 (48 – 104); p < 0.01). No age-related changes in the number of adhering platelets were found in B6 bifurcations (Fig. 3, upper left panel). Conversely, no relation between age and EG thickness was found in the bifurcation of ApoE-/- mice (young: 2.2 (2.0 – 2.7) μm; aged: 2.2 (2.1 – 2.5) μm), while EG thickness significantly increased with age in B6 bifurcations (Young: 2.4 (2.2 – 2.6) μm; aged 3.0 (2.6 – 3.1) μm; p < 0.05; Fig. 3, lower panels).

Atherogenesis

General characteristics of B6 and ApoE-/- mice used are listed in Table 1. Already at seven weeks, ApoE-/- mice showed hypercholesterolaemia and increased levels of LDL compared to B6. In the carotid artery bifurcation of ApoE-/- mice, subendothelial CD11b-positive inflammatory cells were found from the age of 18 weeks onward (Fig. 1c), while plaques were macroscopically visible at the age of 25 weeks and older. In carotid arteries of B6 mice, neither plaques nor CD11b-positive cells were found, as in the common carotids of ApoE-/- mice (not shown).

Table 1: General characteristics of the mouse groups used. B6: wild-type C57Bl6/J mice; ApoE-/-: Apolipoprotein E knock-out mice; young: 7 – 18 weeks; aged: 19 – 31 weeks; LDL: low-density lipoprotein; CCA: common carotid artery. Data are presented as mean ± standard deviation. N-values differ per cell as not all measurements were performed in all animals. Groups were compared using one-way ANOVA with Bonferroni correction. *p ≤ 0.05 compared to aged B6; †p ≤ 0.05 compared to young B6; §p ≤ 0.05 compared to young ApoE-/-.

<table>
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<th>Weight (g)</th>
<th>Plasma cholesterol (mM)</th>
<th>Plasma LDL (mM)</th>
<th>Heart rate (bpm)</th>
<th>CCA diameter (μm)</th>
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<td>Young B6</td>
<td>26 ± 2.1</td>
<td>2.0 ± 0.91</td>
<td>0.2 ± 0.18</td>
<td>255 ± 28</td>
<td>454 ± 41</td>
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<tr>
<td>Young ApoE-/-</td>
<td>27 ± 3.5</td>
<td>9.4 ± 1.61 †</td>
<td>6.3 ± 1.12 †</td>
<td>282 ± 28</td>
<td>460 ± 52</td>
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<tr>
<td>Aged B6</td>
<td>28 ± 1.3 †</td>
<td>1.8 ± 0.35</td>
<td>0.1 ± 0.08</td>
<td>253 ± 42</td>
<td>445 ± 48</td>
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<tr>
<td>Aged ApoE-/-</td>
<td>32 ± 2.4 †5</td>
<td>10.7 ± 2.67 †</td>
<td>7.6 ± 1.89 †5</td>
<td>272 ± 49</td>
<td>452 ± 38</td>
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Discussion

The current study was employed to investigate platelet-vessel wall interactions and EG dimensions during atherogenesis in the same experimental model. To this end, two different microscopy techniques were combined. Data obtained in this way show that the level of platelet adhesion in the carotid bifurcation of ApoE-/- mice is significantly higher, while at the same time the EG at this location is thinner as compared to wild-type controls. This difference in EG dimension between ApoE-/- and control bifurcations appears to be due to an absence of growth of the EG in the hyperlipidaemic animals, while EG thickness increases with age in the controls.

During early atherogenesis, a combination of factors initiates platelet adhesion to the vessel wall, which in turn contributes to local inflammation and subsequent formation of plaques(11, 13–15). The findings of this study indicate that: (a) adhering platelets are mainly found in ApoE-/- mice (effect of mouse strain; Fig. 2); (b) in the carotid bifurcation, significantly more platelets adhere to the wall than in the common carotid artery (effect of vascular location; Fig. 2); and (c) the level of platelet adhesion in the carotid bifurcation of ApoE-/- mice increases significantly with age (effect of age; Fig. 3 upper right panel).

In B6 mice, no age-dependent increase in platelet adhesion is found. At the same time, B6 mice show an age-dependent growth of EG, that is not observed in ApoE-/- mice (Fig. 3). These findings suggest that a lack of EG growth with age in ApoE-/- mice may have contributed to the increase in platelet adhesion at the carotid bifurcation (effect of glycocalyx; Fig. 3). However, the absolute dimension of the EG in the bifurcation of ApoE-/- mice is comparable to that of the common carotid artery in the same animals (Fig. 2), while the level of platelet adhesion differs significantly between both sites. This contradicts a simple and straightforward inverse relation between EG thickness and level of platelet adhesion. We suggest that smaller EG dimensions are not the sole determinant, but that they combine with other site-specific factors, such as flow profiles, to explain the age-dependent increase in pla-
telet adhesion at the carotid bifurcation of ApoE⁻/⁻ mice. In line with this hypothesis, EG reinforcing strategies (26) could help in the prevention of atherosclerosis.

Our data are in line with other publications showing that platelets adhere to atherogenesis-prone sites prior to lesion formation and that damage to the EG further stimulates the formation of atherosclerotic plaques (11, 13, 18). In support of this view, we report a thinner EG in the carotid bifurcation of ApoE⁺⁻ mice than of wild-type controls. In the carotid bifurcation of ApoE⁺⁻ mice, the overall median EG thickness was found to be 2.2 μm. Although significantly lower than in control bifurcations, this EG thickness still largely exceeds the size of adhesion molecules expressed on the endothelium, such as P-selectin (∼0.05 μm) (27, 28). The question arises how platelets can interact with the endothelium despite such a large barrier (even if thinner than in control mice). One explanation may be that platelets have the ability to form very thin membrane extrusions known as tethers, which can reach a length of more than 1 μm (29). Because the EG is estimated to have a low stiffness (30), platelet tethers may easily protrude into the EG to overcome this large obstacle. Other studies describe an increase of EG permeability in the carotid bifurcation of ApoE⁺⁻ mice (8), which might further facilitate platelet tether protrusion into the EG. On the other hand, EG thicknesses as presented in this study represent a central measure derived from a heterogeneous thickness distribution per vessel segment (22). This measure is relatively insensitive to minor changes in local EG composition. In other words, the scanned vessel segments were found to be covered by an EG layer of rather variable thicknesses. Therefore, platelets may not have to overcome the entire reported EG thickness of several micrometers, but may preferentially adhere to endothelial sites covered with thinner EG. Due to the processing of the vessels for ex vivo TPLSM, adhering platelets were not retrieved in the TPLSM images. As a consequence, the relation between the site of platelet adhesion and local EG dimensions is not known. The use of other lectins to label the EG for microscopic imaging might contribute to the identification of thin or more permeable sites in the EG.

We were particularly interested in the relation between EG structure and platelet behaviour during atherogenesis. To enable detailed investigation of changes in both factors, all mice were fed a normal diet. As a consequence, elevated plasma levels of cholesterol and LDL as well as atherosclerotic plaque formation were found in ApoE⁻/⁻ mice only. The fact that platelet- vessel wall interactions occurred more often and at younger ages, and that leukocyte-vessel wall interactions were found common in studies with Western diet-fed ApoE⁺⁻ mice (11, 13) can be ascribed to the accelerated and significantly greater increase in plasma cholesterol levels in these animals (7, 31, 32).

Conclusions

Our data show that in the carotid bifurcation of hyperlipidaemic mice platelet-vessel wall interactions increase significantly in the course of atherogenesis. At the same location, EG growth with age is hampered. Glycocalyx-reinforcing strategies could possibly ameliorate atherosclerosis.

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Conflict of interest

None declared.

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