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Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 Diabetes Mellitus

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Chapter 9

Abstract

Objective: Endothelial glycocalyx perturbation contributes to increased vascular permeability. In the present study, we set out to evaluate whether (i) glycocalyx is also perturbed in DM2 subjects and whether (ii) oral glycocalyx precursor treatment may improve glycocalyx properties.

Methods: Male DM2 patients \(n=10\) and controls \(n=10\) were evaluated before and after 2 months of Sulodexide administration \(200\text{mg/day}\). Glycocalyx dimension was estimated in 2 different vascular beds using Sidestream Dark Field imaging \(\text{SDF}\) and combined fluorescein/ indocyanine green angiography \(\text{FAG/ICG}\) for sublingual and retinal vessels, respectively. Albumin transcapillary escape rate \(\text{TER}\) and hyaluronan catabolism were assessed for vascular permeability.

Results: Both sublingual \(0.64 [0.57 – 0.75] \mu m\) vs. \(0.78 [0.71 – 0.85] \mu m, p < 0.05,\) medians [interquartile range] and retinal glycocalyx dimensions \(5.38 [4.88 – 6.59] \mu m\) vs. \(8.89 [4.74 – 11.84] \mu m, p < 0.05\) were reduced in DM2 compared to controls whereas TER was increased \((5.6 \pm 2.3 \text{ vs con:} 3.7 \pm 1.7 \%), p<0.05\). In line, markers of hyaluronan catabolism were increased in DM2 \(\text{hyaluronan:} 137 \pm 29 \text{ vs} 81 \pm 8 \text{ ng/ml and hyaluronidase:} 78 \pm 4 \text{ vs} 67 \pm 2 \text{ U/ml, both } p<0.05\). Sulodexide increased both sublingual and retinal glycocalyx dimension in DM2 \(\text{sublingual to} 0.93 [0.83 – 0.99] \mu m\) and retinal: to \(5.88 [5.33 – 6.26] \mu m, p<0.05\). In line, a trend towards TER normalization \((\text{to} 4.0 \pm 2.3\%)\) and decreases in plasma hyaluronidase \((\text{to} 72 \pm 2 \text{ U/ml, } p<0.05)\) were observed in DM2.

Conclusion: DM2 is associated with glycocalyx perturbation and increased vascular permeability, which are partially restored following Sulodexide. Further studies are warranted to determine whether longterm treatment may have beneficial effect on cardiovascular risk.
Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 Diabetes Mellitus

Introduction

Diabetes mellitus is characterized by an increased propensity towards vascular complications. Microvascular complications such as retinopathy and nephropathy, as well as macrovascular complications are largely responsible for morbidity and mortality in type 2 diabetes mellitus patients. An early sign of vascular damage consists of increased vascular permeability, which may eventually be associated with microalbuminuria (MA) at a later stage. Although the pathophysiology leading to increased vascular permeability and MA as well as their link with cardiovascular complications has not been fully elucidated, hyperglycaemia is likely to be a causal factor. The endothelial glycocalyx layer consisting of proteoglycans with their associated glycosaminoglycans (GAG’s), covers the luminal side of each vessel wall. Under physiological conditions, the glycocalyx shields the endothelial lining from direct contact with circulating blood elements. Recently, we observed that acute hyperglycaemia results in a profound perturbation of endothelial glycocalyx, which coincided with vascular dysfunction and activation of the coagulation system in healthy volunteers. In fact, with respect to chronic hyperglycaemia, patients with type 1 diabetes mellitus were characterized by a 50% decrease in glycocalyx volume. Loss of glycocalyx volume closely correlated with increased plasma hyaluronidase levels indicative of enhanced hyaluronan catabolism. Glycocalyx perturbation has been associated with a wide spectrum of vascular abnormalities in experimental models, including increased vascular permeability as well as increased adhesion of leukocytes and thrombocytes. In animal models restoration of the glycocalyx reversed these abnormalities. This led to the concept that reversal of glycocalyx damage may be an attractive therapeutic target capable of preserving vascular integrity. In vitro studies have suggested that supplementation of glycocalyx constituents in part restored glycocalyx damage through both increased N-AcetylGlucosamine (GlcNAc) driven GAG synthesis as well as decreased GAG catabolism. Sulodexide is a commercially available compound consisting of heparan sulphate (80%) and dermatan sulphate (20%). Sulodexide is degraded in the digestive tract into GlcNAc building blocks, leading to enhanced precursor abundance for GAG synthesis. In vitro studies have shown that Sulodexide attenuates hyperglycaemia-associated endothelial permeability through enhanced GAG synthesis, but the effect of Sulodexide treatment on diabetes associated microalbuminuria in vivo is less clear. In the present study, we thus hypothesized that enhanced precursor abundance for GAG-synthesis could improve endothelial glycocalyx properties in type 2 diabetes mellitus patients. To this end, we evaluated glycocalyx dimensions in two different vascular beds before and after oral administration of Sulodexide for 8 weeks.
Research design and methods

Study population
In this investigator-initiated study, we enrolled ten non-smoking, male patients with diabetes mellitus type 2 without microalbuminuria (based on 24-hour urine microalbuminuria measurement in the 6 months preceding the study), retinopathy or macrovascular disease (defined as a history of myocardial infarction, stroke, peripheral vascular disease or signs of macrovascular disease at physical examination). All patients used oral antihyperglycemic medication and patients using antihypertensive medication were excluded from participation. Statins were discontinued at least four weeks prior to study initiation. Ten normoglycemic, non-smoking, age-matched healthy male subjects served as an age-matched control group. Participants were asked to refrain from heavy physical exercise 24 hours prior to the study visit. Alcohol, caffeine and metformin were withheld at least 12 hours before the study. All subjects gave written informed consent, and approval was obtained from the internal review board of the Academic Medical Center. The study was registered in the Dutch Trial Register (NTR780/ISRCTN82695186). The study was carried out in accordance with the principles of the Declaration of Helsinki.

Study design
In both patients and age-matched controls we measured 1) local sublingual glycocalyx thickness using Sidestream Dark Field imaging (SDF); 2) retinal glycocalyx thickness using Fluorescein and Indocyanine green angiography (FAG/ICG); 3) transcapillary escape rate of albumin (TERalb); and 4) circulating plasma levels of hyaluronan and its degrading enzyme hyaluronidase both at baseline as well as after 8 weeks of Sulodexide administration (200mg/day; Vessel® 25mg/capsule, Alfa-Wasserman, Milan, Italy). Sulodexide is a glycosaminoglycan of natural origin extracted from mammalian intestinal mucosa, containing a mixture of 80% low-molecular mass heparan sulphate and 20% dermatan sulphate. Blood pressure was measured three times, from which the mean of the last two measurements was used as systolic and diastolic blood pressure.

Endothelial glycocalyx dimension
We assessed the endothelial glycocalyx dimension of both the sublingual as well as the retinal circulation. The determination of the erythrocyte-endothelium gap is the gold standard for glycocalyx measurement in vivo since the endothelial glycocalyx allows limited access to erythrocytes. Using this principle, sublingual glycocalyx dimension was estimated using Sidestream Dark Field (SDF) imaging. Briefly, in each individual approximately 1000 measurement sites of 10 µm long were marked in sublingual vessels. At each measurement site, multiple estimates of the erythrocyte column width were made by measuring both the median red cell width (RBCwidth) as well as the 90th percentile of RBC width distribution. For vessels with diameter classes ranging from 10 – 20 µm, a functional estimate of glycocalyx dimension was estimated by comparing the 50th percentile of RBCwidth with
the 90\textsuperscript{th} percentile of RBCwidth [see figure 1]. Based on these estimates, a single median glycocalyx dimension was calculated for each individual person. The reproducibility of SDF measurement used to estimate glycocalyx dimension has a good reproducibility within our center with an intersession coefficient of variation of 5.6 ± 3.2\% \[n= 10\] controls measured on 2 separate occasions).

**Figure 1.** Sublingual glycocalyx dimension using SDF

The same principle can be applied to retinal endothelial glycocalyx measurement with fluorescence angiography. This method uses the intravascular distribution of two different fluorescent tracers: Fluorescein and Indocyanine green \[18\]. Comparable to the exclusion of erythrocytes, the endothelial glycocalyx also allows limited access to plasma macromolecules, whereas smaller tracers can readily permeate into the glycocalyx \[19\]. In the retinal capillaries, fluorescein fills up the entire vascular compartment while indocyanine green binds to albumin and is therefore excluded by the glycocalyx.
layer. By calculating the difference between the two compartments, the dimension of the glycocalyx layer in retinal vessels can be estimated. Retinal glycocalyx dimensions, represented by the ICG exclusion zone on retinal angiography, were determined in larger retinal vessels (diameter on FAG: 90 µm and above), thereby avoiding the heterogeneity of glycocalyx dimensions in smaller vessels.

**Transcapillary Escape Rate of albumin**

Vascular permeability was determined by the transcapillary escape rate of $^{125}$I-albumin (TERalb) \(^{20}\). $^{125}$I labelled albumin solution of 100 kBq in 5 ml saline was infused as an intravenous bolus. Blood samples were drawn from the contralateral arm at baseline, and at 5, 10, 15, 20, 30, 45 and 60 minutes. Plasma radioactivity was measured in each sample and in a urine sample, collected at the end of the procedure, using a scintillation detector (automatic $\gamma$-counter). TER-alb was expressed as the percentage decline in plasma radioactivity from 10 to 60 minutes after injection.

**Biochemical parameters**

Glucose was assessed using the hexokinase method (Gluco-quant, Hitachi 917; Hitachi). HbA1C was measured by HPLC (Reagens Bio-Rad Laboratories, Veenendaal, the Netherlands) on a Variant II (Bio-Rad Laboratories). Plasma C-reactive protein (CRP) levels were measured with a commercially available assay (Roche, Switzerland). Total cholesterol, HDL-cholesterol, and triglycerides were measured by standard enzymatic methods (Roche Diagnostics, Basel, Switzerland). LDL-cholesterol was calculated using the Friedewald formula. Alanine aminotransferase and aspartate aminotransferase were measured by pyridoxalphosphate activation assay (Roche Diagnostics). Creatinin was measured by Jaffe kinetic colorimetric test (Roche Diagnostics) on Modular P800 (Roche Diagnostics). PT and aPTT coagulation analysis were performed on the Sysmex CA7000 (Siemens healthcare diagnostics, Deerfield, USA). For further analysis, plasma aliquots were snap-frozen and stored at -80°C. Quantitative total plasma hyaluronan levels were measured by enzyme-linked immunosorbent assay (Echelon Biosciences, Salt Lake City, UT) \(^{21}\). Plasma hyaluronidase levels were determined with a previously described assay \(^{10}\).

**Statistical analysis**

Results are expressed as means ± SD. Baseline differences between controls and patients with type 2 diabetes were tested using an unpaired Mann-Whitney test (two-tailed). Differences within groups before and after treatment were tested using a paired Student's t test (two-tailed). CRP and triglycerides were not normally distributed. Therefore, we present medians (interquartile range) and used non-parametric tests for these values. Analyses were performed with SPSS version 11.5 (Chicago, IL, USA). A p value <0.05 was defined as statistically significant.
Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 Diabetes Mellitus

Results

Clinical characteristics

Clinical characteristics of the participants are listed in Table 1. As compared to controls, increased glycemic indices, diastolic blood pressure as well as increased CRP levels characterized patients with type 2 diabetes. No significant changes in BMI, HbA1c or glucose levels were observed upon Sulodexide administration in patients and controls. Besides an increased incidence of epistaxis in one healthy volunteer, there were no other side effects reported.

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Type 2 DM patients</th>
<th>Type 2 DM patients</th>
<th>P*</th>
<th>controls</th>
<th>P**</th>
<th>controls</th>
<th>P***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 wks Sulodexide</td>
<td></td>
<td>Baseline</td>
<td></td>
<td>8 wks Sulodexide</td>
<td></td>
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<tr>
<td>N=10</td>
<td>N=10</td>
<td>N=10</td>
<td></td>
<td>N=10</td>
<td></td>
<td>N=10</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>51.9 ± 8.4</td>
<td>51.9 ± 8.4</td>
<td>P=ns</td>
<td>56.3 ± 8.5</td>
<td>P=ns</td>
<td>56.3 ± 8.5</td>
<td>P=ns</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>5.1 ± 5.6</td>
<td>5.1 ± 5.6</td>
<td>P=ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.7 ± 5.5</td>
<td>29.6 ± 5.3</td>
<td>P=ns</td>
<td>27 ± 2.1</td>
<td>P=ns</td>
<td>26.9 ± 1.8</td>
<td>P=ns</td>
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<tr>
<td>SBP (mmHg)</td>
<td>141 ± 14</td>
<td>139 ± 18</td>
<td>P=ns</td>
<td>135 ± 15</td>
<td>P=ns</td>
<td>141 ± 27</td>
<td>P=ns</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>87 ± 8</td>
<td>84 ± 9</td>
<td>P=ns</td>
<td>82 ± 8</td>
<td>P&lt;0.05</td>
<td>81 ± 6</td>
<td>P=ns</td>
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<td>Glucose (mmol/L)</td>
<td>7.1 ± 2.0</td>
<td>7.3 ± 2.4</td>
<td>P=ns</td>
<td>4.9 ± 0.4</td>
<td>P=0.002</td>
<td>5.2 ± 0.6</td>
<td>P=ns</td>
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<tr>
<td>HbA1c (%)</td>
<td>7.1 ± 1.2</td>
<td>7.1 ± 1.2</td>
<td>P=ns</td>
<td>5.6 ± 0.3</td>
<td>P&lt;0.001</td>
<td>5.5 ± 0.2</td>
<td>P=ns</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.6 ± 1.0</td>
<td>5.3 ± 1.4</td>
<td>P=ns</td>
<td>6.3 ± 1.3</td>
<td>P=ns</td>
<td>5.5 ± 0.9</td>
<td>P=ns</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.4 ± 0.8</td>
<td>3.1 ± 1.3</td>
<td>P=ns</td>
<td>4.4 ± 1.1</td>
<td>P=ns</td>
<td>3.5 ± 1.0</td>
<td>P=ns</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.1 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>P=ns</td>
<td>1.4 ± 0.1</td>
<td>P=0.001</td>
<td>1.4 ± 0.3</td>
<td>P=ns</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.0 [1.4-3.5]</td>
<td>2.1 [1.1-3.0]</td>
<td>P=ns</td>
<td>1.0 [0.9-1.3]</td>
<td>P=0.001</td>
<td>1.3 [0.8-1.4]</td>
<td>P=ns</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.9 [0.5-5.5]</td>
<td>1.5 [0.5-4.4]</td>
<td>P=ns</td>
<td>1.2 [0.5-1.4]</td>
<td>P&lt;0.05</td>
<td>2.6 [0.8-1.4]</td>
<td>P=ns</td>
</tr>
</tbody>
</table>

Values expressed as means ± SD. Triglycerides and CRP are presented as median [interquartile range] due to skewed distribution. BMI indicates body mass index; SBP: systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; TC, total cholesterol; LDL, low-density cholesterol; HDL, High-density cholesterol; TG, triglycerides; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase.

P*: Baseline DM2 patients vs. DM2 Sulodexide
P**: Baseline DM2 patients vs. baseline controls
P***: Baseline controls vs. control Sulodexide

Endothelial glycocalyx thickness measured with SDF and FAG/ICG

Patients with type 2 diabetes mellitus were characterized by reduced sublingual glycocalyx dimensions compared to the controls at baseline: 0.64 [0.57 – 0.75] μm (type 2 diabetes) vs 0.78 [0.71 – 0.85] μm (controls) (p<0.05 type 2 diabetes vs. controls, see Fig 2). Upon Sulodexide, average sublingual glycocalyx thickness increased in diabetic patients (to 0.93 [0.83 – 0.99] μm; p <0.05 placebo vs. sulodexide, see Fig 3). In controls, Sulodexide had no significant effect on glycocalyx dimension (to
0.88 [0.80 – 0.99] µm). In a subset of patients and controls (n=6 per group), retinal glycocalyx dimensions were also determined both before and after 8 weeks of Sulodexide administration. The retinal glycocalyx dimension was reduced in type 2 diabetes mellitus [5.38µm [4.88 – 6.59] vs. controls 8.89µm [4.74 – 11.84], p<0.05 type 2 diabetes vs. controls, see Fig 4]. Following 8 weeks Sulodexide administration, retinal glycocalyx thickness increased in subjects with type 2 diabetes mellitus (5.88 µm [5.33 – 6.26]; p=0.05 vs baseline). In contrast to the effect on sublingual capillary glycocalyx, Sulodexide decreased retinal glycocalyx dimension in controls (to 4.87 [3.89 – 6.33] µm, p<0.05) especially in those with a relatively large glycocalyx dimension at baseline (data not shown).

**Figure 2.** Endothelial glycocalyx in controls and DM2 subjects
(a) Compared to controls, median RBC width increases significantly, resulting in a significant movement of RBCs towards the vessel wall and (b) Glycocalyx dimensions are reduced in DM2 due to significant widening of median RBC width, while both controls and DM2 have similar values of the 90th percentiles of the RBC column width (identifying the position of the vessel wall).
Figure 3. Effect of sulodexide on endothelial glycocalyx in controls and DM2 subjects

(a) Following sulodexide treatment, median RBC width decreases significantly in DM2, resulting in a significant restoration of the distance of RBCs from the vessel wall and (b) following sulodexide treatment, median RBC width is not significantly changed in controls, resulting in similar distance of RBCs from the vessel, (c) Sublingual glycocalyx dimensions are significantly reduced in DM2, and sulodexide treatment restores microvascular glycocalyx dimensions back to control values. Glycocalyx dimension at baseline and after 8 weeks treatment with Sulodexide in controls (n=10, con or Con_Sx) and DM2 (n=10, DM2 or DM2_Sx) and measured by SDF. Individual capillary glycocalyx dimensions are estimated from the transient widening of the erythrocyte column. Data are presented as whisker plots with median [interquartile range].
Figure 4. Effect of sulodexide on retinal glycocalyx in controls and DM2 subjects. Glycocalyx dimension at baseline and after 8 weeks treatment with Sulodexide in controls (n=6, con or Con_Sx) and DM2 (n=6, DM2 or DM2_Sx) measured by FAG/ICG. Individual glycocalyx dimensions are estimated by subtracting the intravascular distribution of two different fluorescent tracers: indocyanine green from fluorescein. Data are presented as whisker plots with median [interquartile range].

Transcapillary Escape Rate of Albumin

TER in the first hour after infusion was increased in type 2 diabetes mellitus patients (5.6 ± 2.3 % in patients vs. 3.7 ± 1.7 % in controls, p <0.05, see Fig 5). Following 2 months Sulodexide administration, a trend towards normalization of TER was observed in type 2 diabetes mellitus (to 4.0±2.3%, p=0.08) whereas in controls TER was unaffected (to 3.3 ± 1.6 %).

Figure 5. Effect of sulodexide on vascular permeability in controls and DM2.

Percentage change in TER-albumin at baseline and after 8 weeks treatment with Sulodexide in controls (n=10, con or Con_Sx) and DM2 (n=10, DM2 or DM2_Sx). Microvascular permeability was determined by the transcapillary escape rate of 125I-albumin (TERalb) between 10 and 60 minutes after infusion. Data are presented as means ± SD.
Biochemistry

Plasma hyaluronan levels were higher in type 2 diabetes mellitus patients compared to healthy controls at baseline (137±29 vs 81±8 ng/ml, p<0.05, see Fig 6). Plasma hyaluronidase levels (indicative of both endogenous hyaluronan- and glycocalyx-degrading capacity) were also increased in patients compared to controls (78±4 vs 67±2 U/ml p<0.05), which is in line with data in type 1 diabetes patients [10,21]. Following Sulodexide administration in type 2 diabetes mellitus, plasma hyaluronidase activity decreased (78±4 to 72±2 U/l, p<0.05) whereas hyaluronan levels were comparable (hyaluronan: 137±29 to 129±15 ng/ml; n.s.). In controls however, hyaluronan (81±8 ng/ml to 107±13 ng/ml, p<0.05) as well as plasma hyaluronidase activity increased (67±2 to 71±3 U/ml, p<0.05) following Sulodexide administration.

Figure 6. Effect of sulodexide on plasma glycosaminoglycans in controls and DM2
Circulating plasma hyaluronan (a) and hyaluronidase (b) levels at baseline and after 8 weeks Sulodexide administration in controls (n=10, con or Con_Sx) and DM2 (n=10, DM2 or DM2_Sx). Data are presented as whisker plots with median [interquartile range].
Discussion

In the present study, we show that endothelial glycocalyx dimension of both sublingual as well as retinal microcirculation is decreased in patients with type 2 diabetes mellitus compared to healthy controls. Following 8 weeks of Sulodexide administration to establish enhanced precursor abundance for GAG-synthesis, glycocalyx dimensions improved in both the sublingual as well as the retinal microvasculature. This improvement coincides with a trend toward improvement of transcapillary albumin escape rate and a reduction in hyaluronan catabolism. These novel findings warrant further studies to evaluate whether endothelial glycocalyx restoration may be of value as an early marker predicting a risk reduction in vascular complication rate in type 2 diabetes mellitus.

Following earlier reports in subjects with type 1 diabetes mellitus, we presently demonstrate the presence of glycocalyx perturbation in two different microvascular beds in patients with type 2 diabetes mellitus. The significant reduction in glycocalyx dimensions in both sublingual as well as retinal vessels in type 2 diabetes underpins the systemic nature of glycocalyx perturbation. The latter coincided with a significant increase in the transcapillary escape rate of albumin indicative of increased systemic permeability lending further support to the concept of systemic glycocalyx perturbation in diabetes mellitus. In this respect, experimental studies have implicated that the endothelial glycocalyx may be instrumental in maintaining the endothelial barrier function under physiological conditions. Alterations in the proteoglycan/glycosaminoglycan-composed matrix-structure of the endothelial glycocalyx could adversely affect the charge-selective repulsion of negatively charged proteins like albumin, thus resulting in increased transvascular leakage in both kidney as well as the systemic vascular barrier in diabetes mellitus. However, systemic albumin clearance (TER) may not be the best method to evaluate changes in systemic vascular permeability, particularly in diabetes mellitus as the impact of impaired reabsorption of albumin in the proximal tubules in diabetes mellitus has been suggested to outweigh potential changes in systemic albumin leakage.

Oral Sulodexide supplementation was associated with a significant improvement of both sublingual and retinal endothelial glycocalyx dimension as well as a trend towards normalization of the TER. Mechanistically, the increased plasma hyaluronan levels and hyaluronidase levels in type 2 diabetes at baseline may reflect increased GAG catabolism in chronic hyperglycaemia, whereas Sulodexide reduced GAG metabolism as attested to by a reduction of plasma hyaluronidase activity. Restoration of GAG metabolism has been suggested to improve the barrier function of the vasculature. Thus, Duling et al previously showed that enhanced abundance of precursors for endothelial GAGs (particularly hyaluronan and unfractionated heparin) led to restoration of the vasculo-protective capacity of the endothelial glycocalyx. In the present study, oral administration of Sulodexide is expected to result in enhanced precursor abundance, which may drive the correction of the endothelial glycocalyx.
low molecular weight heparins may also exert beneficial effects on microalbuminuria and retinopathy in diabetes mellitus 30,31, supplementation of other GAGs delivering comparable molar equivalents of glucosamine, galactosamine and glucuronic acid may share the beneficial properties of Sulodexide on endothelial glycocalyx.

The apparent conflicting results regarding the effect of Sulodexide on microalbuminuria deserve further discussion. The trials reporting a beneficial effect of Sulodexide administration compared to placebo were all performed in renin-angiotensin inhibitor-naive, normotensive type 2 diabetes mellitus patients without overt vascular complications 32-34. Recently, a beneficial effect of Sulodexide was also reported on top of ACE inhibition and/or angiotensin receptor blockers 35. Notably, type 2 diabetes mellitus patients in this trial were relatively young (mean age 55 years) without hypertension and with preserved renal function, and hence less prolonged hyperglycaemic exposure. In contrast, two Sulodexide trials performed in type 2 diabetes mellitus patients aiming to show protective effects of Sulodexide on top of ARBs 36, were terminated prematurely due to lack of an effect on urinary albumin excretion 37. However, patients included in these trials were characterized by hypertension and microalbuminuria, indicating glomerular endothelial damage. Note that the microvascular endothelium may respond differently upon therapeutic intervention compared to glomerular endothelium [as determined by urinary albumin excretion], as they reflect different pathogenic pathways 38-40. In this respect, urinary albumin excretion in diabetes mellitus has been attributed predominantly to proximal tubular dysfunction 41,42, which is independent from changes in glycocalyx properties. Therefore, the lack of a change in urinary albumin excretion in the afore-mentioned trials does not preclude the finding of improved glycocalyx properties in our normalbuminuric patients. In addition, the time scales may profoundly affect the observed results. Thus, vascular barrier function restoration may follow at a later stage when the endothelial glycocalyx is already restored 12,13. To definitively answer the question whether early intervention with Sulodexide is able to prevent the development of diabetes-associated microvascular damage, a trial comparing Sulodexide to placebo in newly diagnosed normotensive/normalbuminuric patients with type 2 diabetes mellitus is warranted.

Mechanistically, the impact of Sulodexide on glycocalyx-dimension and properties in health and disease is a challenge 15. Endothelial glycocalyx thickness depends on the rate of synthesis, the rate of shedding as well as the circulating levels of GAG-degrading enzymes, including hyaluronidase and heparanase 43,44. Surprisingly, an increase in sublingual glycocalyx dimension with a concomitant decrease in retinal glycocalyx was observed following Sulodexide administration in controls. These data imply that endothelial responses are heterogeneous not only in patients with type 2 diabetes versus controls, but also in various vascular beds 45. Apparently, endothelial glycocalyx dimension is more closely regulated in the retina than the sublingual microvessels 46. The driving force behind glycocalyx synthesis is most likely determined by enhanced precursor abundance, comprising glucosamine, galactosamine and glucuronic acid. Following intestinal degradation of orally administered Sulodexide,
increased levels of GAG-precursors may contribute to the increased plasma levels of hyaluronan and hyaluronidase in controls. In diabetes mellitus however, GAG-degradation is increased 10,21. In these patients, decreased plasma hyaluronidase activity may be attributed to the inhibition of enzymatic glycocalyx degradation by the (Sulodexide-derived) increased substrate bioavailability 12. In support, Sulodexide has been hypothesized to inhibit heparanase (and possibly hyaluronidase) activity, both involved in GAG catabolism 47. Therefore, in line with the pioneering study by Potter et al 46, future in vitro endothelial glycocalyx studies will have to focus on the effect of GAG supplementation on the regulation of GAG degrading enzymes in different vascular endothelium (glomerular, capillary and retina).

This study has several limitations. First, the dose of Sulodexide used was not validated by a formal dose-finding study. Previous studies have indicated that a treatment period as long as 6 months may be required to establish the maximal effect 48. This delayed mode of action virtually precludes performing a meaningful dose-finding study. Therefore, we selected the 200 mg Sulodexide dose based predominantly on previously published data 35. This data has shown improvement in the endothelial barrier function using comparable concentrations of Sulodexide in vitro 16. Note that the anticoagulant effects of heparan sulphates, potentially leading to bleeding complications, may also, at some point, limit further dose escalation. Second, our present proof-of-concept study was rather small, whereas only type 2 diabetes patients without cardiovascular and/or proteinuric disease were included. Whereas the findings are potentially interesting, they need further confirmation in a larger cohort as well as in type 2 diabetes patients with more severe stages of vascular complications.

In conclusion, subjects with type 2 diabetes mellitus are characterized by a clear perturbation of the endothelial glycocalyx layer, which is thought to reflect increased vascular vulnerability. Oral Sulodexide administration improves endothelial glycocalyx dimension to the same extent in two different vascular beds [sublingual and retinal], most likely due to enhanced precursor abundance for GAG-synthesis following Sulodexide administration. Improvement of glycocalyx dimension coincided with a trend toward normalized systemic vascular permeability and GAG metabolism. Collectively, the present findings imply that restoration of endothelial glycocalyx in humans may be a promising target to attenuate vascular dysfunction in type 2 diabetes mellitus. Further research is required to evaluate which treatment modalities are most to establish improvement in endothelial glycocalyx dimension. More importantly, prospective studies should address whether the concept holds true that endothelial glycocalyx improvement predicts long-term cardiovascular benefit in diabetes mellitus.
References


[37] ClinicalTrials.gov identifier: NCT00130312 and NCT00342238

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