

# The relation between altered shear stress-mediated mechanotransduction and loss of glomerular endothelial glycocalyx in the development of albuminuria

**Bachelor thesis**  
*Pathophysiology*

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## **Abstract**

Albuminuria is a pathological condition, which is characterised by an elevated urinary albumin excretion. Under physiological conditions, the glomeruli are able to prevent albumin passage through the glomerular filtration barrier. An important structure that contributes to albumin retention is the glomerular endothelial glycocalyx. The glycocalyx is a gel-like layer that is localised on the luminal side of glomerular endothelial cells. However, damage to the endothelial glycocalyx leads to passage of albumin through the glomerular wall, which may result in albuminuria.

Besides its barrier function, the endothelial glycocalyx also acts as a mechanosensor. Mechanosensors on endothelial cells provide sensing of dynamic blood flow (shear stress), in which mechanotransduction can be induced. Mechanotransduction provides intracellular signalling that is required for regulation of endothelial cell homeostasis. Loss of glycocalyx might result in altered shear stress-mediated mechanotransduction. In addition, impaired mechanotransduction might lead to loss of glycocalyx. Therefore, altered shear stress-mediated mechanotransduction possibly results in albuminuria. This thesis aims to explore the possible relation between altered shear stress-mediated mechanotransduction and loss of glomerular endothelial glycocalyx in the development of albuminuria.

Loss of the endothelial glycocalyx is characterised by degradation of heparan sulphate and hyaluronan glycosaminoglycans within the glycocalyx layer. Disturbed shear stress (disturbed blood flow) and disturbed shear stress-induced mechanotransduction can provoke degradation of both glycosaminoglycans. On the other hand, laminar shear stress (normal blood flow) and laminar shear stress-induced mechanotransduction prevent degradation of the glycocalyx.

In addition, the endothelial glycocalyx itself also contributes to mechanosensing. However, loss of glycocalyx leads to a decreased blood flow-sensitivity, which causes impaired shear stress-induced mechanotransduction. Subsequently, altered mechanotransduction reinforces degradation of heparan sulphate and hyaluronan. Loss of glycocalyx leads to increased permeability for albumin through the glomerular filtration barrier, which contributes to the development of albuminuria.

In conclusion, loss of glomerular endothelial glycocalyx, associated with albuminuria, can be a cause and/or consequence of altered shear stress-mediated mechanotransduction.

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## Introduction

Albuminuria is a pathological condition in which an abnormal level of albumin is found in the urine. An increased protein concentration of albumin in the urine indicates that the renal blood filtering function is deteriorated [1]. Therefore, protein composition of urine is a general marker to detect possible renal dysfunction in clinical practice. Albuminuria is divided into two categories: micro- and macroalbuminuria. Microalbuminuria is diagnosed when urinary albumin level is 30-300 mg per day [2]. For macroalbuminuria, the protein level is above 300 mg per day [3]. Both types can occur in patients with diabetes mellitus, hyperglycaemia, heart failure, hypertension and/or kidney failure [4]. In addition, several studies proposed that albuminuria may also cause progression of cardiovascular events and kidney disease [4-8]. Therefore, this suggests that treatment of albuminuria is a potential intervention [8].

Albumin is the most abundant protein in blood. This protein is negatively charged due to lysine, arginine, glutamic acid and aspartic acid side chains and has a molecular weight of 66,5 kDa [9]. Albumin plays a vital role in the body, because this protein is a carrier for different substances, such as hormones, enzymes and drugs in the circulation [9]. Furthermore, one of the most important functions of albumin is to maintain a constant blood volume. Because of its relative high molecular weight, negative charges and high concentration in the blood vessels, albumin attracts water from surrounding tissues and avoids loss of water through the vascular wall. Increase of water in the circulation results in a low blood viscosity, which is favourable for blood pressure and flow [9].

Under physiological conditions, blood plasma consists of 55-60% albumin [9]. Per day, a maximum of 70 kg of albumin can reach the kidney [9]. It is generally known that the kidney filters blood to maintain homeostasis of the internal environment [10]. This filtration leads to production of urine. Under physiological circumstances, the capillary beds in glomeruli are able to impede passage of albumin to renal tubules. It is possible that a small amount of albumin enters the tubules, but the major part is reabsorbed in other parts of the kidney [10]. These processes provide that urinary albumin excretion is below 30 mg per day. Nevertheless, glomerular function can decrease, which leads to increased albumin permeability. Subsequently, this increased permeability can result in albuminuria.

An important structure which may contribute to development of albuminuria is the endothelial glycocalyx [11]. The glycocalyx is a gel-like coating on the luminal surface of all endothelial cells. Especially in the glomeruli, this coating is important for restricting passage of albumin in glomerular capillaries. However, it was found that damage of the glomerular endothelial glycocalyx causes an increased permeability for albumin [12], which suggests a contribution to the development of albuminuria.

Apart from its barrier function, the endothelial glycocalyx is also known as possible mechanosensor of shear stress in blood vessels [13]. A normal blood flow exerts a mechanical shear stress on endothelial cells, which is sensed by mechanosensors [14]. Consequently, these mechanosensors induce mechanotransduction. Mechanotransduction is a mechanism to transmit mechanical forces through endothelial cells into biochemical stimuli. These biochemical stimuli provide activation of several signalling pathways to regulate endothelial cell homeostasis [15]. This regulation is required for maintaining a proper function and survival of endothelial cells. Because the glycocalyx is blood flow-sensitive [13], it is possible that loss of glycocalyx results in attenuated mechanosensing, which may lead to an impaired mechanotransduction. In addition, impaired mechanotransduction may cause deteriorated regulation of endothelial cells and, therefore, possibly leads to more damage to the glycocalyx. This process suggests that altered shear stress-mediated mechanotransduction contributes to development or reinforcement of albuminuria.

Therefore, it might be possible that loss of glomerular endothelial glycocalyx, associated with albuminuria, is a cause and/or consequence of altered shear stress-mediated mechanotransduction. This thesis aims to explore a possible link between mechanotransduction-associated damage to the glomerular endothelial glycocalyx and the development of albuminuria. To achieve this, the properties of the glomerular glycocalyx and the effects of mechanotransduction on glycocalyx and vice versa will be investigated.

# Chapter 1: The glomerular filtration barrier

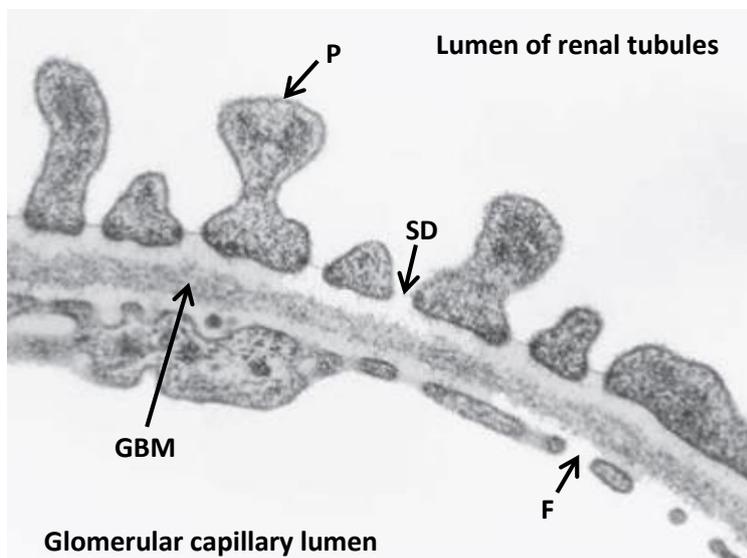
Production of urine is essential to maintain homeostasis. The glomerulus of the kidney provides the first step in urine production. The glomerulus is composed of a network of glomerular capillaries. To filter waste products, electrolytes and water from the bloodstream, the glomerulus contains a glomerular filtration barrier [16]. This barrier provides transcapillary transport from glomerular capillaries to renal tubules. In the tubules, filtered water and substances are further processed to achieve an average of 1 litre of urine per day [10]. The glomerular filtration barrier ensures that water easily passes the capillary wall of glomeruli. Also, relatively small, water-soluble substances are capable to flow effortlessly through this barrier. However, the glomerular filtration barrier ensures exclusion of passage of macromolecules. This semi-permeability is dictated by the size and charge of molecules; the larger and more negatively charged a molecule is, the more difficult it is to flow through the glomerular wall [10].

## 1.1 Structural components of the glomerular filtration barrier

The selectivity of the glomerular filter is caused by specific structural components, such as endothelial cells, the glomerular basement membrane and podocytes (figure 1) [16]. The capillaries are lined with endothelial cells. Endothelial cells in glomeruli are highly fenestrated [17], which means that glomerular capillaries contain pores. The size of a fenestration in glomeruli is 60-80 nm in diameter [18]. The size of albumin is 3.8 nm in diameter [19], which is smaller than endothelial fenestrations. Yet, albumin is the best known plasma protein for its restricted passage through glomeruli under physiological circumstances [10]. This restriction is due to fixed negative charges in fenestrations to repel plasma proteins [20]. These negative charges in fenestrations are mainly caused by the glomerular endothelial glycocalyx (chapter 2) [8].

The layer of endothelial cells lies on a basal membrane, the basement membrane, which also contributes to semi-permeability of glomeruli (figure 1) [16]. Glomerular basement membrane is mostly composed of collagen and proteoglycans, which give the basement membrane a negative charge as well [16].

Lastly, podocytes are structured as foot-like processes to surround glomerular capillaries [21]. Like endothelial fenestrations and the basement membrane, podocytes also contain negative charges. Between two podocytes, a gap can be seen, which means that podocytes are not continuously surrounding each capillary (figure 1). This gap is termed the glomerular slit diaphragm and its structure allows flow of glomerular filtrate to renal tubules [22]. Thus, each component of the glomerular filtration barrier contributes to production of glomerular filtrate [10].



**FIGURE 1.** Transmission electron micrograph of the glomerular filtration barrier of a rat *ex vivo*. The glomerular wall consists of fenestrated endothelial cells, a basement membrane and podocytes to regulate permeability of the glomerular wall. Arrows indicate: *GBM* glomerular basement membrane, *P* podocyte, *SD* slit diaphragm, *F* fenestration. Magnification: approximately 48000X. Adapted from Satchell et al. [18].

## Chapter 2: The glycocalyx

As mentioned above, the endothelial glycocalyx is a particular component within the glomerular filtration barrier, which contributes to albumin retention [23]. The glycocalyx is a luminal coat for endothelial cells of all vascular beds [24]. The main structure of the endothelial glycocalyx is formed by two groups of molecules, glycoproteins and proteoglycans (figure 2) [12].

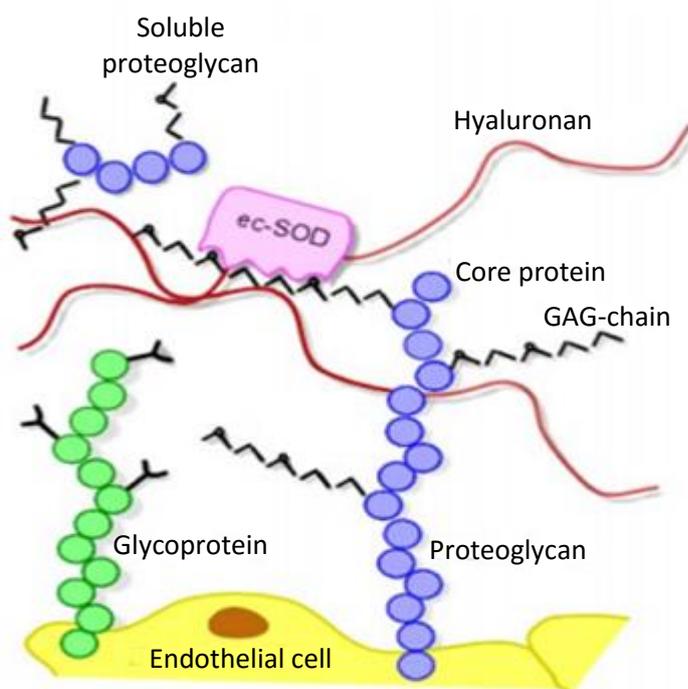
### 2.1 Glycoproteins

One of the major components of the endothelial glycocalyx is the glycoprotein (figure 2). Glycoproteins are proteins, which are modified by the addition of covalently attached small, branched carbohydrates [11]. The synthesis and modification of glycoproteins occur in the endothelial cell [11]. Furthermore, glycoproteins in the glycocalyx are connected to the plasma membrane of endothelial cells and have a variety of functions in the vasculature, such as mediating leukocyte adhesion during inflammation [25-27].

### 2.2 Proteoglycans

The endothelial glycocalyx is also composed of proteoglycans (figure 2). Proteoglycans contain core proteins, e.g. syndecans and glypicans [28,29], which are covalently attached to a variable number of glycosaminoglycans [11]. Examples of glycosaminoglycans, which are prominently abundant in the endothelial glycocalyx, are heparan sulphate and chondroitin sulphate [30-32]. Both glycosaminoglycans have undergone post-translational modifications, such as sulfation [11]. These sulfations cause anionic charges, which explain the observed fixed negative charges in the fenestrations of endothelial cells, as described earlier.

Another important glycosaminoglycan in the endothelial glycocalyx is hyaluronan. Hyaluronan differs from heparan sulphate and chondroitin sulphate. Instead of binding at core proteins, hyaluronan is attached to binding protein CD44 at the endothelial surface [33]. Furthermore, hyaluronan lacks sulfation [8]. Nevertheless, it attracts water molecules to give the glycocalyx its hydrated, gel-like structure [11].



**FIGURE 2. Structure of the endothelial glycocalyx.**

The endothelial cells are covered with the glycocalyx. In the glycocalyx, proteoglycans are attached to the endothelial cell membrane. Proteoglycans consist of core proteins, which are bounded to glycosaminoglycans (GAG). These glycosaminoglycans can bind soluble substances, such as hyaluronan and extracellular superoxide dismutase (ec-SOD), which are derived from the circulation and/or endothelial cells. Hyaluronan is a glycosaminoglycan and ec-SOD is an enzyme, which functions as an antioxidant. In the glycocalyx, also glycoproteins are present, which are attached to endothelial cells. The glycoproteins also bind soluble substances. Adapted from Reitsma [11].

### *2.3 Glomerular endothelial glycocalyx prevents albumin passage*

In glomerular endothelial cells, it has been observed that the glycocalyx is also located between fenestrations and not only on the endothelial surface [8]. Hyaluronan anchors the glycocalyx to the basement membrane of the glomerular filtration barrier [34]. Therefore, fenestrations are not empty spaces [8]. Due to its structure and molecular properties, the luminal side of the glycocalyx in glomerular capillaries is also able to trap soluble substances, such as proteins and proteoglycans [11]. As a consequence, the glycocalyx acts as a mediator for blood plasma- and vessel wall homeostasis [35]. In addition, the negatively charged albumin in the circulation can also be trapped in the glycocalyx [36], which reinforces the negative charge of the layer. Moreover, the proteoglycans and glycoproteins cause steric hindrance with its negative charges and glycosaminoglycan fibers. These properties prevent albumin from passing through the glomerular filtration barrier [11].

### *2.4 The glycocalyx as a mechanosensor*

Importantly, the endothelial glycocalyx is a mechanosensor on endothelial cells [13]. Thus, the glycocalyx is blood flow-sensitive. Sensing of blood flow can affect albuminuria. The underlying process of mechanosensing and its role in albuminuria will be described in chapter 3.

### *2.5 Attenuated albumin restriction*

In 1976, Ryan and Karnovsky provided new insights in distribution of albumin in the glomerular filtration barrier of rats during different haemodynamic conditions (normal and abnormal blood flow) [16]. They localised albumin in kidneys of different experimental groups. In the first group of rats, normal blood flow was maintained. For two experimental groups, ligation of renal vein and/or artery was performed to completely stop blood flow through the kidney. For each group, ligation was maintained for 5 minutes. Lastly, a fourth group was used to investigate albumin distribution after restoration of blood flow. This was done by performing ligation of renal artery and vein for 5 minutes. Subsequently, both ligations were removed to restore blood flow for another 10 minutes. After surgical procedures, all rats were directly fixated *in situ*. Thereafter, albumin was stained with immunoperoxidase reaction in tissue sections of kidneys to localise protein expression in different compartments of the glomerular filtration barrier.

Under normal blood flow, it was found that albumin was highly concentrated in the glomerular capillaries and endothelial fenestrations, but in the glomerular basement membrane and lumen of renal tubules there was no staining. In the groups of ligated rats, clumped albumin products were found in the basement membrane and lumen of tubules. In rats with restored blood flow, albumin localisation was the same as in rats with normal blood flow [16]. This study suggested that blood flow restriction (or the ligation procedure) caused an increased albumin permeability in glomeruli. In addition, restoration of blood flow might recover albumin exclusion. Therefore, alterations of blood flow might be associated with the development of albuminuria.

The question arises if blood flow plays a role in loss of albumin retention by affecting the glomerular endothelial glycocalyx. As mentioned in Chapter 2.4, the endothelial glycocalyx acts as a mechanosensor that is sensitive for blood flow. Moreover, the endothelial glycocalyx mainly provides albumin retention in the glomeruli. As a consequence of altered blood flow, the glomerular filtration barrier is not able to impede albumin passage. Therefore, this generates the hypothesis that loss of glycocalyx is associated with impaired blood flow.

## Chapter 3: The glycocalyx responses to shear stress

### 3.1 Blood flow and shear stress

The glomerular endothelial glycocalyx is exposed to blood flow. Moreover, blood flow in the glomeruli is regulated by arterioles of the kidney to maintain metabolic needs. The glomerulus is located between the afferent and the efferent arteriole, which both can constrict. Vasoconstriction of the afferent arteriole leads to decreased flow in the glomerular capillaries. However, constriction of the efferent arteriole results in an increased pressure in the glomeruli. As a consequence, renal blood flow and pressure are dynamic (between 80 and 170 mmHg) [10].

In all blood vessels, blood flow exerts a mechanical force at the luminal surface of the wall. The mechanical force per unit area is called shear stress. Shear stress regulates endothelial cell morphology, gene expression and function [37]. Because the velocity of blood flow is dynamic and changes under physiological and pathological conditions, endothelial cells are subjected to altered shear stress.

In the case of the vasculature of the kidney, dynamic blood flow ensures that the shear stress in glomerular capillaries is variable. According to mathematical calculations, the extreme values of shear stress are between 1 and 96 dynes/cm<sup>2</sup>, but the most observed values are between 5 and 20 dynes/cm<sup>2</sup> [38].

### 3.2 Laminar shear stress and mechanotransduction

Unidirectional laminar shear stress (normal blood flow) within blood vessels is sensed by mechanosensors, which are expressed on endothelial cells [39]. In response to shear stress, these mechanosensors convert mechanical stimuli to intracellular biochemical signals to the nucleus of the cell. This process of mechanosensing and transmission of signals is called mechanotransduction [14]. This shear stress-induced mechanotransduction upregulates activity of endothelial NO synthase (eNOS), which leads to increased production of nitric oxide (NO) in the endothelial cell [37]. NO, also known as an endothelium-derived relaxing factor [40], is an important molecule, which regulates vascular homeostasis; e.g. NO increases vasodilatation, decreases expression of leukocyte adhesion molecules at endothelial cells and inhibits coagulation [41-43].

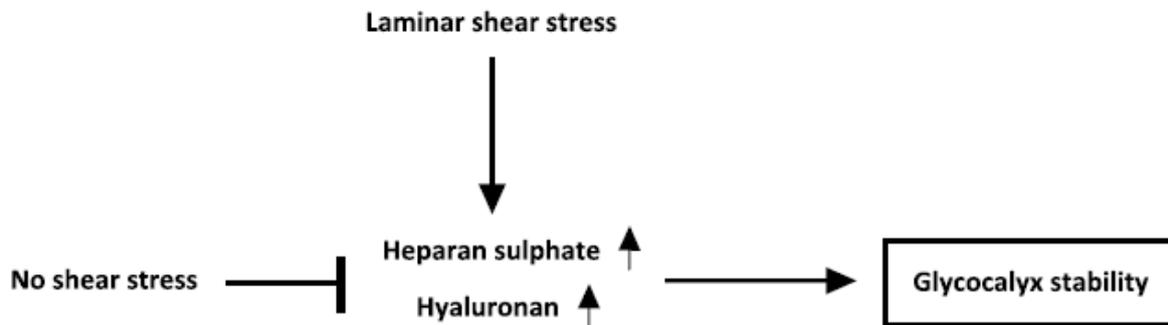
### 3.3 Laminar shear stress and the endothelial glycocalyx

It has been observed that laminar shear stress can affect the glycocalyx composition. Researchers have shown that laminar shear stress provided a glycocalyx thickness of  $399 \pm 174$  nm in murine carotid artery. However, exposure to disturbed shear stress caused a significant reduction in thickness ( $73 \pm 36$  nm) [44]. A decreased thickness was a consequence of loss of glycocalyx. Thus, alteration of blood flow results in a dynamic thickness of the endothelial glycocalyx.

In addition, several studies investigated the effects of laminar shear stress on glycosaminoglycan synthesis in comparison with altered or no shear stress. For example, Arisaka et al. [45] observed enhanced production of heparan sulphate in cultured aortic porcine endothelial cells after exposure to steady, laminar shear stress for 24 hours compared to static control (no shear). Other researchers, Gouverneur et al. [46], investigated the effects of shear stress on hyaluronan in human umbilical vein endothelial cells (HUVEC). They showed a threefold increase of hyaluronan in the endothelial glycocalyx when exposed to shear stress for 24 hours. In a follow-up experiment, Maroski et al. [47] showed that laminar shear stress increased mRNA and protein levels of hyaluronan synthase compared to no shear. Hyaluronan synthase is able to produce hyaluronan glycosaminoglycans.

Thus, previously described studies showed that laminar shear stress provided hyaluronan and heparan sulphate synthesis. Besides that, laminar shear stress also resulted in an increased thickness

of the endothelial glycocalyx compared to static control or disturbed shear stress. Therefore, laminar blood flow has advantageous effects on the composition of the glycocalyx (figure 3). Unfortunately, it is not known if this also applies to glomerular endothelial glycocalyx.



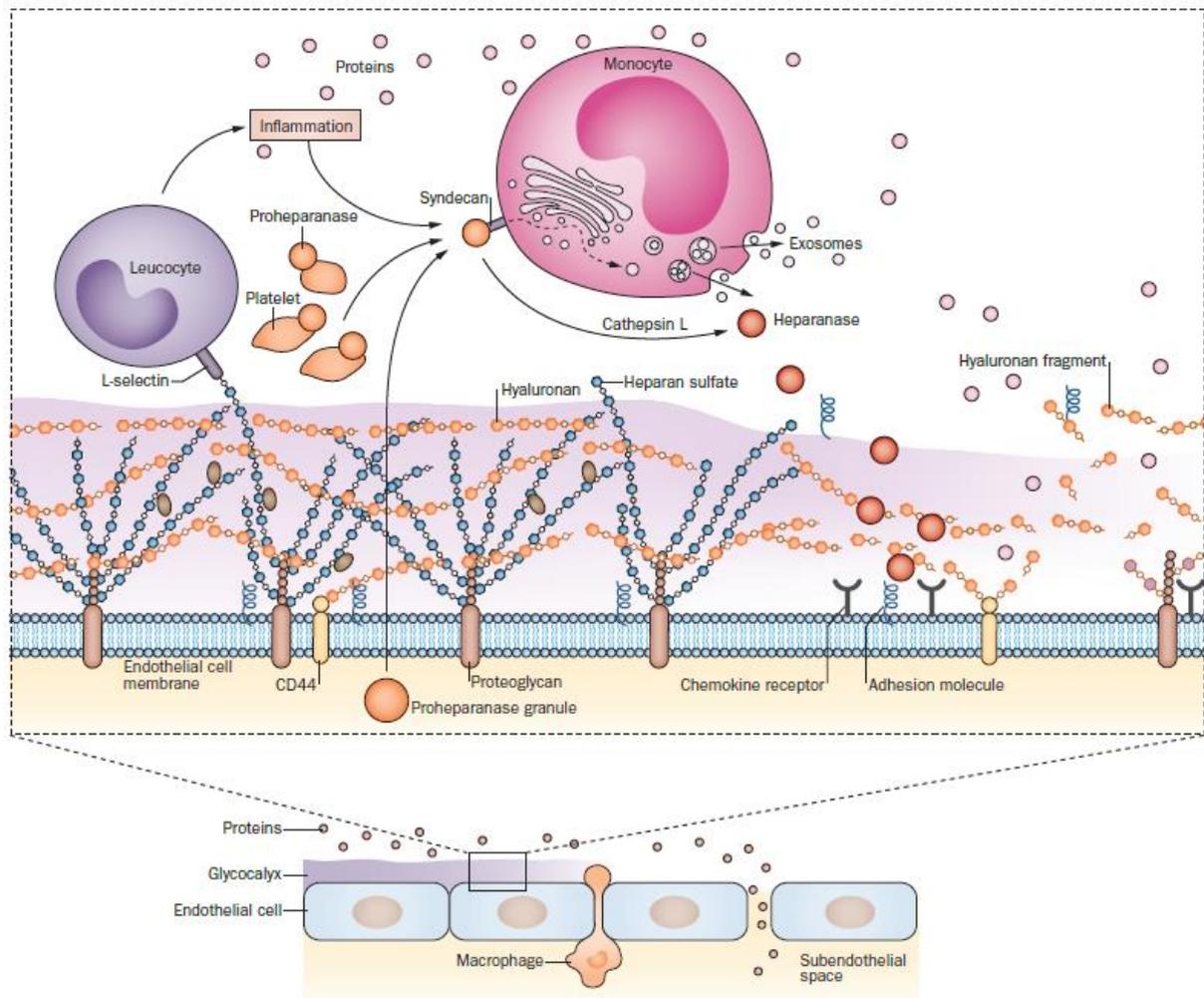
**FIGURE 3. A hypothetical pathway from laminar shear stress to glycocalyx stability.** Laminar shear stress provides increased heparan sulphate and hyaluronan synthesis compared to no shear stress [45-47]. In addition, disturbed shear stress reduces the thickness of the endothelial glycocalyx compared to laminar shear stress [44]. Therefore, this suggests that the glycocalyx stability is being maintained by laminar shear stress.

### 3.4 Disturbed shear stress and mechanotransduction

Besides normal blood flow, disturbed blood flow may also occur. Disturbed flow occurs in curved and branched blood vessels, but also in pathological conditions, such as hypertension, diabetes mellitus and ischemia-reperfusion [37]. Furthermore, disturbed flow alters shear stress-mediated mechanotransduction. In contrast to laminar shear stress, disturbed shear stress leads to decreased NO production. Subsequently, NO cannot exert its homeostatic, anti-inflammatory effects during disturbed shear stress [37].

### 3.5 Endothelial activation and the endothelial glycocalyx

Disturbed shear stress leads to endothelial activation (as reviewed in [48]), which alternatively causes damage of the endothelial glycocalyx. As a consequence of sustained and strong endothelial activation provoked by disturbed shear stress, heparan sulphate is modified differently during synthesis (figure 4) [8]. These chemical modifications (6-O sulfation and N-deacetylation) result in binding domains for chemokines on heparan sulphate [49,50]. A consequence is that chemokines bind to the glycocalyx [8]. Subsequently, chemokines attract leukocytes from the circulation, whereafter L-selectin on leukocytes bind to heparan sulphate [51]. These processes provoke an inflammatory response. Simultaneously, endothelial activation also causes release of proheparanase and hyaluronidase (figure 4) [52,53]. It is known that proheparanase is released by endothelial cells, blood platelets and leukocytes during endothelial activation (figure 4). Furthermore, the inflammatory response induces monocytes and macrophages to secrete cathepsin L [54]. Cathepsin L cleaves proheparanase to its active conformation, namely heparanase. Heparanase is able to degrade heparan sulphate glycosaminoglycan in the glomerular endothelial glycocalyx and hyaluronidase is an enzyme which degrades hyaluronan [53]. The mechanism to release hyaluronidase is not known, but it has been found that activity of hyaluronidase is upregulated during endothelial activation. Therefore, endothelial activation leads to loss of glycocalyx components in the glomeruli due to degradation of hyaluronan and heparan sulphate [8]. As a consequence, the endothelial glycocalyx will be damaged, which results in deteriorated albumin restriction.



**FIGURE 4. Endothelial activation and glycocalyx damage.** Endothelial cells, leukocytes and platelets release proheparanase due to endothelial activation. Simultaneously, endothelial activation leads to cathepsin L secretion by monocytes and macrophages [54]. Cathepsin L ensures conversion of proheparanase into heparanase. Heparanase causes degradation of heparan sulphate in the endothelial glycocalyx. Besides that, hyaluronidase is also released during endothelial activation [53]. Hyaluronidase causes degradation of hyaluronan in the glycocalyx, which leads to circulating hyaluronan fragments. Degradation of heparan sulphate and hyaluronan results in loss of the endothelial glycocalyx. From Rabelink and De Zeeuw [8].

As described above, endothelial activation leads to production of heparan sulphate chemokine binding domains, in which heparan sulphate become degraded. It is known that this modification of heparan sulphate is induced by N-deacetylase-N-sulfotransferase-1 (Ndst) enzyme. Rops et al. [50] performed a knockdown study in mice to investigate whether activation of Ndst leads to albuminuria. They showed that knockdown of Ndst resulted in a low reduction of albuminuria in mice compared to control. Thus, degradation of heparan sulphate might contribute to albuminuria.

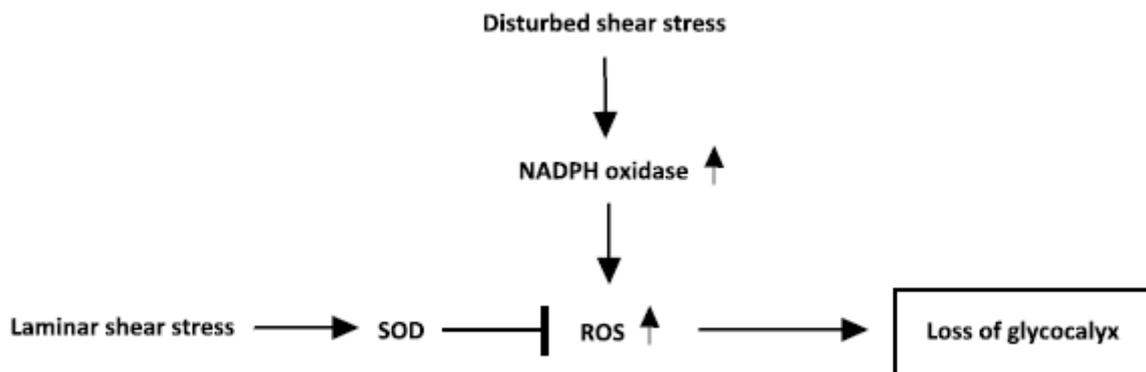
### 3.6 Reactive oxygen species and the endothelial glycocalyx

Besides endothelial activation, disturbed shear stress also causes elevated reactive oxygen species (ROS) levels. Disturbed shear stress induces activation of NADPH oxidase, which produces ROS, such as superoxide ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) [55]. Subsequently, ROS reacts with NO to produce peroxynitrite ( $ONOO^-$ ) [56], which leads to a decreased NO level.

Laminar shear stress also increases ROS by activation of NADPH oxidase, but the shear stress simultaneously increases activity of cytosolic copper zinc-containing superoxide dismutase (Cu/Zn

SOD) [37]. Cu/Zn SOD allows  $O_2^-$  scavenging, which ensures lower ROS levels and prevents loss of NO. However, disturbed shear stress does not upregulate SOD activity. Therefore, NO cannot exert its homeostatic, anti-inflammatory effects during disturbed shear stress [37]. In addition, elevated ROS cause damage to endothelial cells [37]. Therefore, ROS due to disturbed shear stress may influence the endothelial glycocalyx [8].

Indeed, in glomeruli it has been shown that ROS can damage the endothelial glycocalyx. Singh et al. [57] have investigated the effects of  $H_2O_2$  exposure to cultured glomerular endothelial cells of human. They showed a significant decrease of heparan sulphate on the cell surface when exposed to  $H_2O_2$ . In addition, this decrease was not observed when  $H_2O_2$  was added in combination with SOD and other ROS scavengers. Furthermore,  $H_2O_2$  did not influence the morphology and survival of the endothelial cells. Therefore, ROS have a considerable effect on loss of glomerular endothelial glycocalyx by degradation of heparan sulphate (figure 5). Moreover, because laminar shear stress induces SOD activity, it suggests that laminar shear stress inhibits ROS-induced loss of glycocalyx (figure 5).



**FIGURE 5. A hypothetical pathway from disturbed shear stress to loss of glycocalyx due to reactive oxygen species (ROS).** Disturbed shear stress provokes increased ROS production via activation of NADPH oxidase compared to no shear stress. ROS are able to degrade heparan sulphate, which leads to loss of glycocalyx [57]. Laminar shear stress induces superoxide dismutase (SOD) activity, which is a ROS scavenger [37]. Therefore, laminar shear stress may prevent loss of glycocalyx by reducing ROS levels.

### 3.7 Endothelial glycocalyx acts as mechanosensor

Currently, it is known that altered shear stress and mechanotransduction-mediated ROS can affect composition of the endothelial glycocalyx. As described earlier, shear stress is sensed by mechanosensors on endothelial cells, which is followed by mechanotransduction. Laminar shear stress-induced mechanotransduction provides increased NO production and disturbed shear stress attenuates this process. Additionally, the endothelial glycocalyx acts as one of the mechanosensors to participate in blood vessel homeostasis [13]. Therefore, it suggests that the glycocalyx also regulates mechanotransduction.

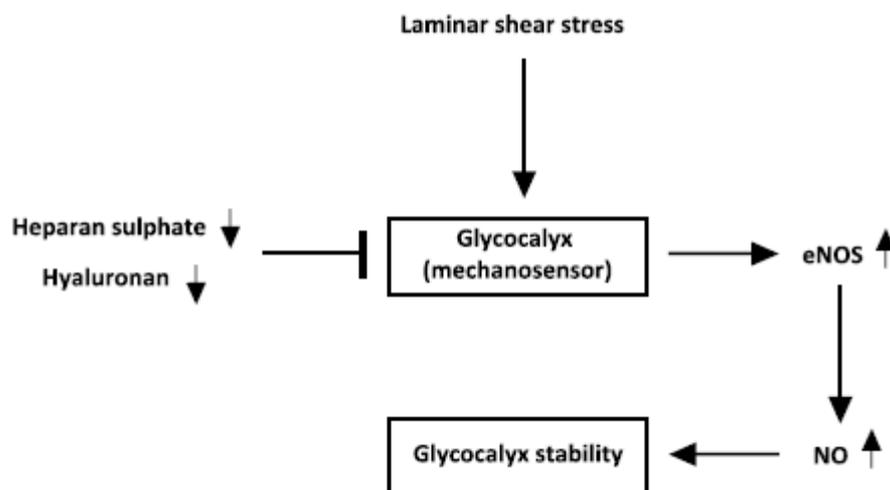
In several studies, it has been observed that the endothelial glycocalyx also mediated NO production in response to shear stress. Florian et al. [58], for example, investigated shear stress-induced NO production on cultured bovine aortic endothelial cells (BAEC), in which heparan sulphate glycosaminoglycan in the glycocalyx was degraded after treatment with the enzyme heparanase. Cells with and without degradation of heparan sulphate were exposed to steady and disturbed shear stress for 180 minutes. For both types of shear stress, NO production was significantly lower after degradation of heparan sulphate. Moreover, the production of NO was almost completely abolished without heparan sulphate. Therefore, this suggests that heparan sulphate is an important component of the endothelial glycocalyx to mediate NO production.

Pahakis et al. [59], have done a similar study as Florian et al., but also investigated other glycosaminoglycans. They used heparanase, chondroitinase and hyaluronidase to degrade respectively heparan sulphate, chondroitin sulphate and hyaluronan in the glycocalyx of cultured BAECs. Subsequently, the cultured cells were exposed to steady shear stress (20 dynes/cm<sup>2</sup>) or no shear stress (static control) for 180 minutes. The researchers showed in endothelial cells with enzyme treatment that NO production was significantly decreased after steady shear stress compared to its static control. Under steady shear stress conditions, NO production was significantly decreased in endothelial cells after enzyme treatment for hyaluronan and heparan sulphate, but this was not observed after treatment for chondroitin sulphate. So, the researchers suggested that hyaluronan was also an important component to mediate NO production, like heparan sulphate.

Yen et al. [60] have performed *in vivo* experiments in rat mesenteric blood vessels to investigate flow-induced NO production during heparanase treatment. Heparanase treatment caused a complete inhibition of flow-induced NO production. Furthermore, the researchers investigated eNOS activity by using N<sup>G</sup>-monomethyl-L-arginine (L-NMMA), which inhibits eNOS. It was observed that L-NMMA caused a decrease of flow-induced NO production without heparanase treatment. Therefore, Yen et al. suggested that flow-induced NO production mediated by the glycocalyx was due to increased eNOS activity.

Previous studies indicated that the loss of glycocalyx due to reduced heparan sulphate and/or hyaluronan led to impaired NO production [58-60]. In addition, heparan sulphate and hyaluronan in the endothelial glycocalyx might detect and amplify shear stress for mechanotransduction [11]. Shear stress was sensed and transduced by the glycocalyx to increase NO production via upregulation of eNOS activity. Moreover, glycocalyx damage due to degradation of hyaluronan or heparan sulphate resulted in an attenuated sensing of shear stress, in which NO production was decreased. However, it is not known if this mechanism is also found in glomerular endothelial cells.

In addition to glycocalyx-mediated mechanosensing, shear stress and NO are both important for the maintenance of the composition of the glycocalyx [8]. Hence, impaired mechanotransduction that is caused by loss of glycocalyx may reinforce damage to the endothelial glycocalyx. Therefore, even if there is normal shear stress, loss of glycocalyx may start a vicious circle.



**FIGURE 6. A hypothetical pathway from laminar shear stress to glycocalyx stability as a consequence of glycocalyx-mediated mechanosensing.** The glycocalyx acts as a mechanosensor that can sense blood flow. Laminar shear stress causes glycocalyx-mediated mechanotransduction, which activates endothelial nitric oxide synthase (eNOS). This activation results in elevated nitric oxide (NO) production [58-60]. NO maintains or reinforces the glycocalyx stability [8]. Degradation of heparan sulphate and hyaluronan leads to disturbed mechanosensing in the glycocalyx, which leads to decreased NO production.

### *3.8 Loss of endothelial glycocalyx, steric hindrance and albuminuria*

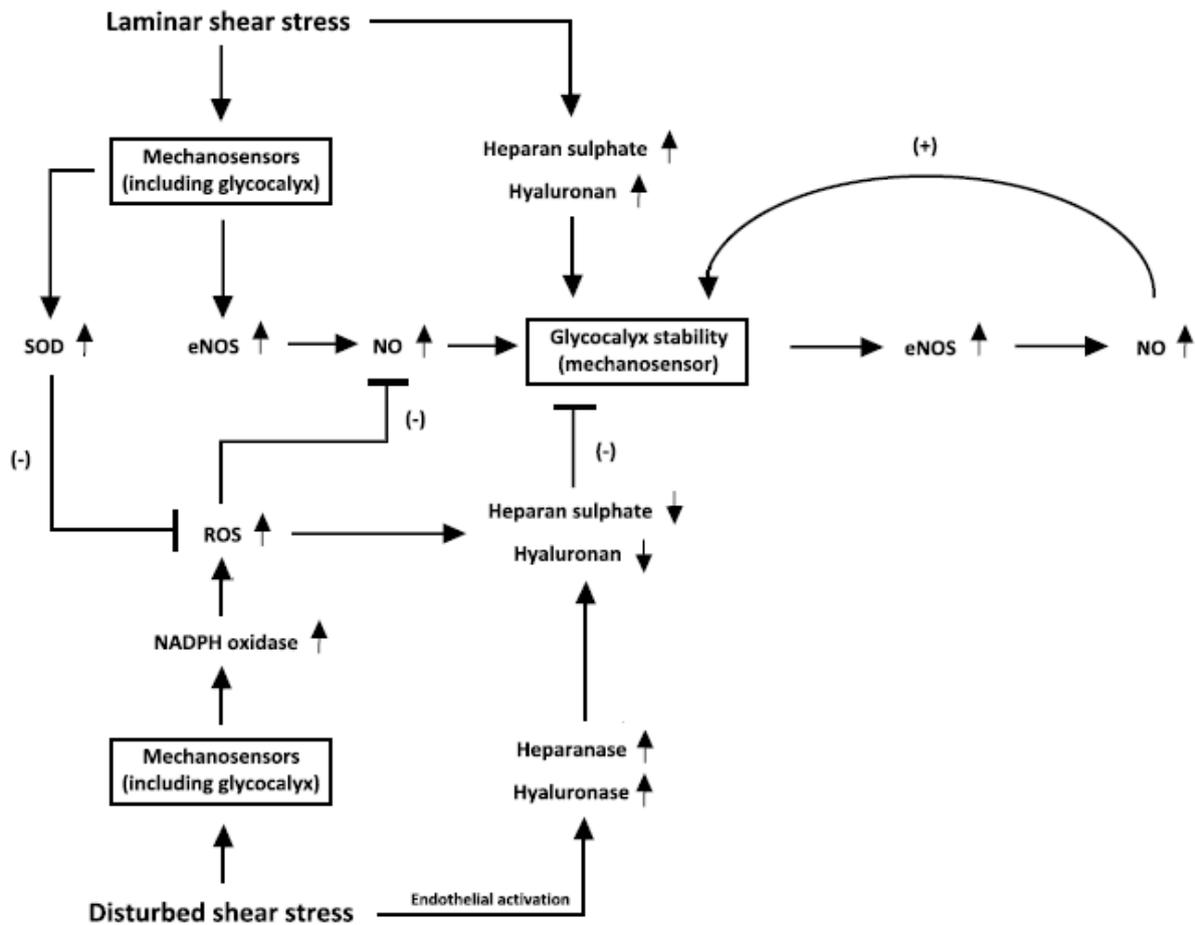
As discussed above, altered shear stress-mediated mechanotransduction is associated with loss of glycocalyx components. In addition, loss of glycocalyx is associated with loss of its steric hindrance. Ueda et al. [61] performed a study in which the effects of shear stress and steric hindrance on albumin passage through the endothelial glycocalyx was been investigated. They observed in cultured BAECs that removal of negative charge in the glycocalyx with protamine sulphate led to an increased permeability for albumin under static conditions (no shear). Moreover, the researchers showed that exposure of disturbed shear stress caused an even higher permeability compared to no shear stress. On the other hand, laminar shear stress resulted in a decrease of albumin passage with 76% compared to no shear stress. Overall, each type of shear stress had different effects on the restriction of albumin in the endothelial glycocalyx. Moreover, loss of glycocalyx reinforced this effect [61]. However, this study did not specifically involve glomerular endothelial cells.

Nevertheless, there is evidence that loss of glomerular endothelial glycocalyx leads to albuminuria. As described earlier, Ryan and Karnovski [16] showed that the glomerular filtration barrier did not allow albumin passage during normal blood flow. However, blood flow restriction provoked albumin passage. It is currently known that the endothelial glycocalyx mainly impedes albumin passage. On the other hand, it has been observed that degradation of heparan sulphate and hyaluronan caused passage of albumin through the glomerular filtration barrier [34,62].

In addition, Boels et al. [63] investigated whether restoration of the glomerular endothelial glycocalyx had an effect on albumin passage in mouse models for diabetic nephropathy. Diabetic nephropathy is an albuminuria-associated disease and is also characterised by a decrease in thickness of the endothelial glycocalyx [64]. During the experiment [63], mice were treated with atrasentan, which is a drug that causes increased NO production. As described earlier, NO provides glycocalyx stability. The researchers showed that heparanase activity was inhibited due to atrasentan treatment. Moreover, atrasentan also reduced albuminuria and restored the glomerular endothelial glycocalyx, which was comparable with control mice. In addition, they showed that atrasentan treatment also caused an increased thickness of the endothelial glycocalyx in HUVECs in the same study. Therefore, these observations suggested that increase of glycocalyx thickness resulted in a reduction of albuminuria. Conversely, loss of glycocalyx contributed to the development of albuminuria.

## Discussion and conclusion

The aim of this thesis was to explore the relation between altered shear stress-mediated mechanotransduction and loss of glomerular endothelial glycocalyx in albuminuria. Ligation of the renal vasculature causes attenuated glomerular barrier function, which results in albumin permeability. Currently, it is known that the endothelial glycocalyx is an important structure within the glomerular filtration barrier to impede albumin passage. In particular, heparan sulphate and hyaluronan glycosaminoglycans contribute to albumin restriction. Unfortunately, loss of glomerular endothelial glycocalyx may lead to albuminuria.



**FIGURE 7. Integration of shear stress-mediated mechanotransduction and glycocalyx stability.** Several hypothetical pathways lead to reinforcement of the glycocalyx stability due to laminar shear stress-mediated mechanotransduction. The endothelial glycocalyx acts as a mechanosensor. As a result of laminar shear stress, the glycocalyx provides an increased NO production. NO reinforces the endothelial glycocalyx, which improves albumin restriction through the glomerular filtration barrier. On the other hand, several hypothetical pathways lead to degradation of the endothelial glycocalyx due to disturbed shear stress. As a consequence, loss of glycocalyx leads to attenuated albumin restriction, which may cause albuminuria. Moreover, damage to the endothelial glycocalyx may induce impaired glycocalyx-mediated mechanosensing. This results in less NO production, which further leads to damage to the glycocalyx. Hence, disruption of the glycocalyx may cause a vicious circle.

Several studies, as mentioned in this thesis, provided better understanding in the link between blood flow and the endothelial glycocalyx (as depicted in figure 7). To start with, dynamic blood flow provokes different types of shear stress on endothelial cells. Laminar and disturbed shear stress both have different effects on the composition of the endothelial glycocalyx. Laminar shear stress provides synthesis of heparan sulphate and hyaluronan. This synthesis leads to an increased thickness of the

endothelial glycocalyx, which contributes to restriction of albumin. In contrast to laminar shear stress, disturbed shear stress causes a decreased thickness of the endothelial glycocalyx. Moreover, disturbed shear stress leads to endothelial activation. Endothelial activation induces increased heparanase and hyaluronase activity, which results in degradation of heparan sulphate and hyaluronan. Overall, dynamic blood flow can affect the composition of the endothelial glycocalyx.

Additionally, shear stress is also related to mechanotransduction. Endothelial cells contain mechanosensors that are sensitive for blood flow. Presumably, all mechanosensors work together to initiate blood flow-induced mechanotransduction. Mechanosensing provoked by laminar shear stress results in elevated NO and decreased ROS levels for maintaining blood vessel homeostasis. However, disturbed shear stress provokes decreased NO and increased ROS levels. ROS exposure to the endothelial glycocalyx leads to degradation of heparan sulphate, which contributes to loss of the endothelial glycocalyx. Moreover, laminar shear stress may prevent this degradation by inducing SOD activity, which leads to ROS scavenging. Therefore, altered shear stress-mediated mechanotransduction also plays an important role in regulation of the endothelial glycocalyx composition.

Subsequently, the endothelial glycocalyx acts as a mechanosensor. Sensing of laminar shear stress via the glycocalyx provides upregulation of eNOS activity, which leads to increased NO production. In addition, Florian et al. [58] and Pahakis et al. [59] showed that enzymatic degradation of heparan sulphate and/or hyaluronan resulted in attenuated glycocalyx-mediated mechanosensing. As a consequence, NO production was reduced. Moreover, it is known that disturbed shear stress can provoke degradation of heparan sulphate and hyaluronan, which lead to loss of glycocalyx. This suggests that disturbed shear stress can lead to deteriorated sensitivity for blood flow in the glycocalyx.

Moreover, NO mediates the glycocalyx composition. It has been shown that upregulation of NO due to atrasentan led to an increased thickness of the endothelial glycocalyx. In addition, the glycocalyx provides NO production as a consequence of laminar shear stress. Therefore, disturbed shear stress or loss of glycocalyx can both lead to more damage to the endothelial glycocalyx, which may result in altered shear stress-mediated mechanosensing.

Lastly, loss of glycosaminoglycans results in a decreased thickness of the endothelial glycocalyx. Moreover, loss of glycosaminoglycans is also associated with reduced steric hindrance. As a consequence, albumin can pass the glomerular filtration barrier. In addition, treatment of albuminuria by increasing the glycocalyx thickness provides reduction of albuminuria. This suggests that loss of glycocalyx is associated with albuminuria.

In clinical practice, albuminuria is a marker to indicate renal dysfunction. However, several studies proposed that albuminuria is also a cause of progression of renal disease, which suggests that treatment of albuminuria is a potential intervention [8]. To investigate this, clinical trials were conducted that attempted to reduce albuminuria with several drugs [8]. These studies showed that albuminuria was decreased, but this decrease was not associated with diminished development of new cardiovascular and kidney diseases. Therefore, it was concluded that treatment of albuminuria is not a potential intervention to improve cardiovascular and renal function. According to Rabelink and De Zeeuw [8], however, this conclusion is arguable, because the drugs used in the clinical trials did not target the primary cause of albuminuria, which is presumably loss of endothelial glycocalyx. For this reason, Rabelink and De Zeeuw suggested that clinical trials should be performed with drugs that are associated with the primary reduction of albuminuria, such as drugs that can promote restoration of the glycocalyx. Before this is possible, a better understanding in loss of glycocalyx and albuminuria is required. To achieve this, the functions of the glycocalyx need to be clarified.

This thesis provides an integration of the glycocalyx as a barrier for albumin passage and as a mechanosensor. Several components of the hypothetical pathway (figure 7) are potential targets to decrease loss of glycocalyx or reinforce the stability of the endothelial glycocalyx. As a consequence, albuminuria may decrease and progression of renal and cardiovascular diseases may be prevented.

Unfortunately, no previous studies have been done to determine the effects of shear stress alterations on mechanotransduction in glomerular endothelial cells. Therefore, further research is required to determine if altered shear stress and impaired mechanotransduction actually occur in glomeruli. Nevertheless, disturbed shear stress as the primary cause of loss of glycocalyx should not necessarily be the case. Other causes of glycosaminoglycan degradation, such as high glucose [65], also lead to instability of the glycocalyx, which may result in impaired glycocalyx-mediated mechanosensing. This impaired mechanosensing may lead to altered mechanotransduction. Even if there is a normal shear stress, altered mechanotransduction can lead to loss of glycocalyx, which finally leads to attenuated restriction of albumin. This process can contribute to the development of albuminuria

In conclusion, the suggested link between shear stress-mediated mechanotransduction and the endothelial glycocalyx is that loss of glomerular endothelial glycocalyx, associated with albuminuria, is a cause and/or consequence of altered shear stress-mediated mechanotransduction.

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