Albuminuria: more than a renal risk marker?

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Introduction and Aims of Thesis

Albuminuria, more than a marker of renal damage?

Albuminuria is the excretion of the plasma protein albumin in urine. Urinary albumin is absent or low in healthy conditions. In the last decades, large cohort studies have shown that mildly increased levels of albuminuria (microalbuminuria; urinary albumin concentration > 20 mg/L or 24-hr excretion > 30 mg) are present in healthy subjects (5-8%) and highly prevalent in patients with diabetes (18-39%) and hypertension (11-23%).[1-8]

Interestingly, albuminuria has been reported as a risk marker for renal and cardiovascular disease: the higher the albumin in urine, the higher the risk for renal and/or cardiovascular disease.[9-15] This immediately raises the question whether we should lower albuminuria to preserve function of these organs. However, the importance of albuminuria as a therapeutic target in renal and cardiovascular disease is under discussion. In order to accept a biomarker, in this case albuminuria, as an appropriate therapeutic target, three important criteria should be met.[16,17] First, evidence of a consistent and independent association between levels of albuminuria and renal/cardiovascular end points during follow-up must be available. Second, clinical trial evidence needs to show an association between the effects of different interventions that lower albuminuria and the subsequent reduction of renal and cardiovascular risk. And third, a biological mechanism should be available to explain how albuminuria causes renal damage.

Indeed, cohort studies have shown a strong, consistent and independent association between the amount of albuminuria and risk of renal and cardiovascular disease, both in healthy subjects and in patients with diabetes or hypertension, meeting the first criterion for the acceptance of albuminuria as a therapeutic target.[9-15] Moreover, lowering of albuminuria has been shown to slow down renal disease progression. This has been investigated in multiple post-hoc analyses, and for all these studies the same paradigm holds: the greater the reduction in albuminuria during the first months of therapy, the larger the reduction of renal or cardiovascular risk during subsequent follow-up.[18-22] The biological mechanism that explains the association between albuminuria and renal damage has been much debated. Initially, it was hypothesized that albuminuria is a consequence of renal damage. Indeed, when the glomerular filter in the kidney is damaged, increased amounts of albumin could pass the glomeruli and leak into the urine. However, recently evidence has been provided that albuminuria may be more than only a marker of kidney damage, and could be a cause of kidney damage. The relatively recently discovered glycocalyx (a layer covering the endothelium) appears to be a barrier for albumin to leak out of the vasculature, including the glomerulus (Figure 1).[23] This sheds a different light on the mechanism of increased renal leakage of albumin, since the first barrier appears to be not the glomerular basement membrane and slit pores, but the vessel wall itself. Second, if albumin leaks into the primary urine, the podocytes, mesangium and tubules are exposed to high concentrations of albumin. Proximal tubules are equipped with protein reabsorption transporters, through which also albumin is

retrieved, reabsorbed and then degraded in the proximal tubular cell. In physiological circumstances, low quantities of albumin may be reabsorbed and degraded. However, when high quantities appear in the primary urine, albumin reabsorption may activate pro-inflammatory substances in the tubular cell changing the tubule's phenotype and leading to interstitial damage, fibrosis, and ultimately reduced nephron function (Figure 2).[24-33]

An explanation for the relationship between albuminuria and risk of cardiovascular disease has been proposed by the Steno hypothesis, which states that excessive urinary albumin loss is the result of widespread peripheral vascular damage or endothelial dysfunction.[34] According to this hypothesis albumin leakage is not confined to the kidney, but is present throughout the peripheral vasculature. Several studies using radioactively labeled albumin, indeed demonstrate that microalbuminuria is associated with an increased permeability of the peripheral vascular beds to macromolecules.[35-37] Again, this is linked to the glycocalyx, which is present in vasculature throughout the whole body.

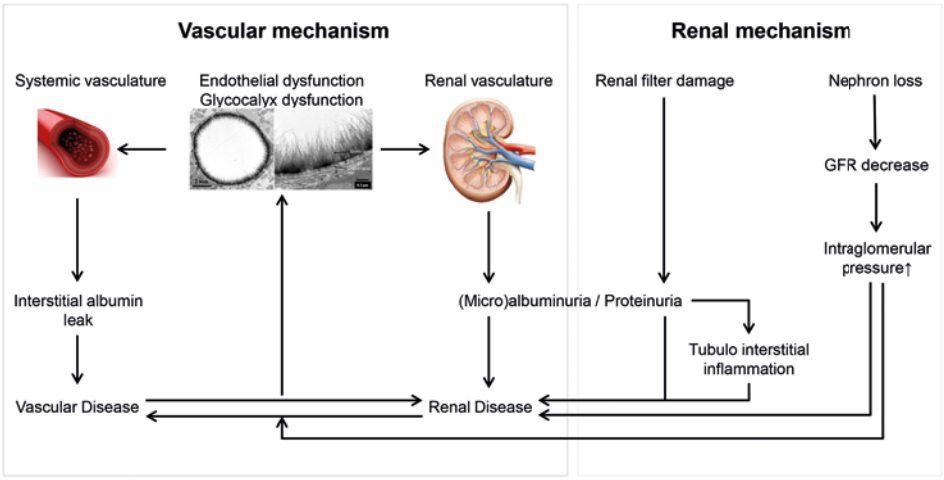

Figure 1. Link between vascular and renal mechanisms causing vascular and renal disease

If indeed albuminuria is a cause of renal and/or cardiovascular disease, the question arises: "what causes albuminuria to increase?" Is microalbuminuria a condition acquired during life, as a result of western lifestyle (e.g. high salt and protein intake), smoking, hypertension or diabetes, leading to vascular wall damage? Or is albuminuria a congenital condition? Clinical studies have shown that high albuminuria levels precede and predict new-onset hypertension and diabetes, rendering the first possibility less likely. This would mean that elevated levels of albuminuria might be an inborn characteristic. A mechanism explaining why albuminuria would already be present at birth has been defined in the Barker hypothesis. This hypothesis states that already in the womb, fetuses may be harmed due to genetic factors, malnutrition and other insults to the mother during pregnancy.[38]

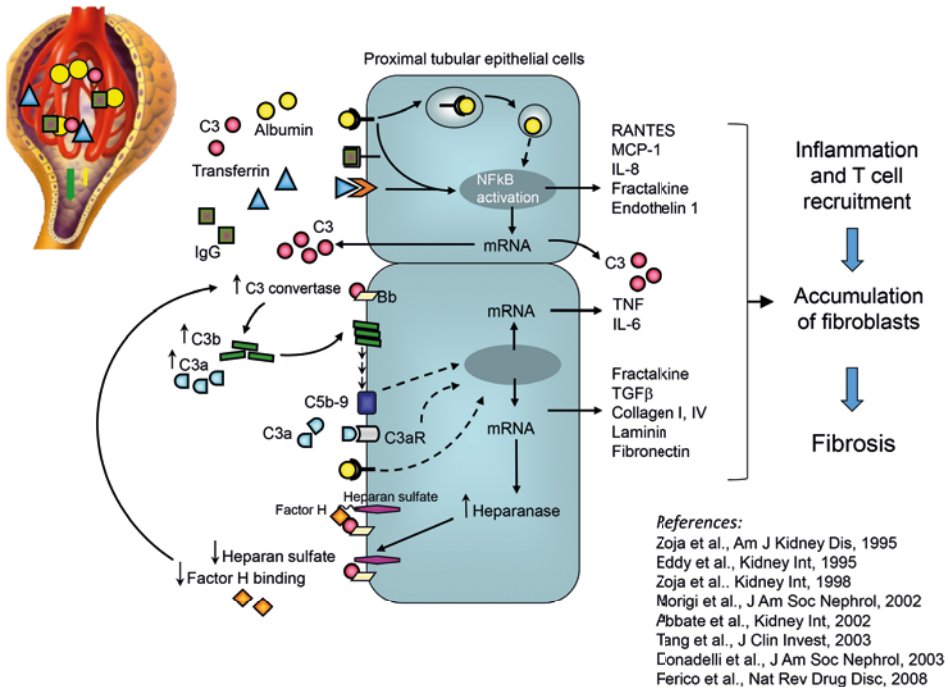

Figure 2. Mechanisms underlying the activation of inflammatory and fibrogenic pathways in proximal tubular epithelial cells by ultrafiltered protein load.[26-33]Adapted from Abbate et al. JASN 2006

If albuminuria is already present at birth, this would make the use of albuminuria as a target even more important as it would facilitate early identification of individuals at risk of organ function loss and it would create the possibility to intervene early in the disease process. A shift could take place from intervention to prevention of disease. However, there is a paucity of data on albuminuria in toddlers and young children, emphasizing the importance of further research in this area. Data on the association between albuminuria and renal endpoints and the effect of treating albuminuria in children are lacking. Moreover, guidelines for the optimal methods for measuring and monitoring albuminuria in children are absent. Therefore, more research into different aspects of albuminuria in children is needed.

Measurement of albuminuria

In order to correctly identify and monitor the level of albuminuria in an individual, one has to measure albuminuria in the most optimal way. In clinical practice, a wide variety of urine collection strategies are being used. One can perform a 24-hour urine collection, collect a first morning void urine sample or a random urine sample (anytime during day or night). A single sample (on a single day) or multiple samples (multiple subsequent or

separate days) can be used and one can measure either the albumin concentration, or use the albumin:creatinine ratio to correct for hydration status. In adults, multiple studies have investigated and compared different urine collection strategies in order to find the optimal strategy that introduces the least bias.[39-43] Currently, the gold standard in adults is still the 24-hour urinary albumin excretion rate, although a good and practical alternative is the measurement of the albumin:creatinine ratio in first morning void samples (average of three consecutive days).[44] There are no clear guidelines available in children, since no exhaustive studies on optimal urine collections and measurements in children have been conducted.

A problem in assessing the level of albuminuria in young children is the choice of the urine collection method itself. Babies and toddlers are incontinent for urine. This makes the collection of urine for albumin measurements cumbersome. Modern diapers are extremely efficient in retaining urine, thus old fashioned cotton wool or modern pantyliners can be used to help recovering urine. This technique is not only unhandy in use but also irreproducible.[45,46] Taped plastic bags are frequently used as an alternative in clinical practice, but these have the disadvantage that they detach easily from the skin and appear to be uncomfortable.[45] What is the optimal urine collection method for albuminuria assessment in young children, which is practical in use, reliable and comfortable still needs to be established. This optimal urine collection method, in combination with better guidelines on urine collection strategies for albuminuria assessment in children would improve the quality of further research on the importance of albuminuria as a potential renal and cardiovascular risk marker in children.

Treatment of albuminuria

Based on the experimental and clinical data showing that albuminuria might be a causal factor in renal and cardiovascular disease development, albuminuria seems an independent target for renal and cardiovascular protective therapy. This is important in the context of new intervention strategies to halt the progression of renal disease. Currently, several therapies for the reduction of albuminuria are available.

The most important therapy used in current clinical practice, which has been clearly shown to delay renal disease progression, is Renin-Angiotensin-Aldosterone-System (RAAS) inhibition with Angiotensin Converting Enzyme (ACE) inhibition or Angiotensin Receptor Blockade (ARB). The exact mechanism by which RAAS inhibition lowers albuminuria is still unknown. Inhibition of the RAAS system (RAASi) lowers the pressure of the efferent arteriole of the glomeruli, thereby reducing the intraglomerular pressure and the glomerular filtration rate. This could lead to changes in the vascular barrier and a reduction of the filtration of molecules like albumin over the glomerular membrane. However, the haemodynamic effects of RAASi are nearly instantaneous and stay stable after that initial effect, whereas albuminuria lowering takes weeks to stabilize.[47,48] Secondary effects of this intraglomerular pressure fall on the integrity of the glomerular

barrier function may apply. Also effects of RAASi on the glycocalyx can play a role.[49,50] Whatever the relation between RAASi and albuminuria lowering is, it is clear that RAASi protects the kidney and that this protection appears to be associated with the lowering of risk factors among which albuminuria.

Although there is overwhelming randomized controlled clinical trial evidence available in adults demonstrating that RAAS inhibition delays the progression of renal function decline, a debate continues arguing that in certain settings RAASi is not efficacious and even unsafe. [51-57] An example is a recent publication that advocates to stop RAASi in patients with advanced renal disease. The haemodynamic fall in eGFR after initiation of RAASi has been shown to be reversible after discontinuation.[58] With this in mind, it has been advocated to discontinue RAASi in patients with late stage chronic kidney disease in order to regain renal function and delay dialysis.[56] However, the consequence of this discontinuation has not been properly studied.

In contrast to the large amount of data on the renal protective effect of RAAS inhibition in adults, randomized studies in children are lacking. The ESCAPE study, in which all children were treated with standardized ACE inhibition showed that ACE inhibition was effective in lowering of proteinuria with an average initial reduction in proteinuria of 50%.[59] In the observational CKiD study, ACE inhibitor use was associated with lower proteinuria levels.[60] Use of an ARB was shown to be more effective in lowering of proteinuria than a calcium channel blocker in a small randomized open-label study.[61] Two small uncontrolled studies demonstrated stabilisation of kidney function in children with CKD that were treated with an ACE inhibitors or ARB.[62,63] Finally, one observational study failed to show evidence that ACE inhibition slowed the progression of CKD.[64] Both the effect of RAAS inhibition and the discontinuation of this treatment on renal disease progression are unknown and need to be further established.

Aims of this thesis

This thesis aims to investigate different aspects of albuminuria in children, in order to increase the knowledge on this subject and enable future research in this area. It focuses on the prevalence, measurement and treatment of albuminuria.

Part 1. Prevalence and measurement of albuminuria in children.

Part 1 begins by investigating whether microalbuminuria is already present in young children without kidney disease. **Chapter 2** describes the prevalence of microalbuminuria in toddlers from an observational cohort study in the Northern part of the Netherlands, and compares the distribution of albuminuria levels with an adult cohort of the general population from the same area. Moreover, associations between the presence of microalbuminuria and different antenatal, postnatal and maternal factors are tested.

In **Chapter 3,** the optimal urine collection method for assessment of albuminuria in toddlers has been investigated. As very young children are not continent for urine, urine collection in this population is an issue. The method used in chapter 2 is compared with a newly development method, the PeeSpot and a reference method, i.e. an unprocessed urine sample which is stored at 4°C.

Chapter 4 studies the most optimal strategy for measuring and monitoring albuminuria in toddlers in an observational prospective cohort study. Different urine collection strategies are tested and compared with the gold standard in adults, which is the measurement of urinary albumin:creatinine ratio in three first morning void samples, collected over time.

Part 2: Treatment of albuminuria in children

Part 2 of this thesis focuses on the treatment of albuminuria in children. In **Chapter 5**, the initial effect of the ACE inhibitor ramipril on proteinuria and its association with renal disease progression are tested in 280 children with Chronic Kidney Disease (CKD) in a post-hoc analysis of the Effect of Strict Blood Pressure Control and ACE inhibition on the Progression of Chronic Renal Failure in Pediatric Patients (ESCAPE) trial. It was also determined whether residual proteinuria (i.e. the proteinuria level during ACE inhibition) is associated with a higher renal risk. Combined, these measurements study the importance of proteinuria as a therapeutic target in the management of children with CKD.

Chapter 6 assesses the effect of discontinuation of RAAS inhibition therapy on important renal markers and renal disease progression in an observational cohort study of children with CKD, in order to investigate whether discontinuation of RAAS inhibition for clinical reasons is indeed indicated. Moreover, it evaluates the most important reasons for discontinuation of RAAS inhibition in a clinical practice setting.

This thesis ends with a summary of the results of the conducted studies and an overview of future perspectives.

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Part 1

The prevalence and measurement of albuminuria in children