EDUCATIONAL REVIEW

Microalbuminuria: causes and implications

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Abstract Management strategies are increasingly focused on tackling the increasing burden of cardiovascular disease worldwide. Microalbuminuria is a powerful predictor of cardiovascular disease and mortality in adults. This holds true in the general adult population but is particularly recognized in those with diabetes, where it identifies those likely to develop progressive atherosclerotic vascular disease and renal impairment. The atherosclerotic process begins in childhood with likely consequences in later life. In-depth understanding of the mechanisms through which microalbuminuria occurs holds promise for designing therapies to arrest its development in the future. Microalbuminuria arises from increased leakage of albumin through the complex glomerular sieve known as the glomerular filtration barrier. This requires changes in the physio-chemical properties of components of this barrier. However, the increased glomerular permeability confirmed in disease does not necessarily correlate with recognized histological changes in the glomerulus, suggesting that perhaps more subtle ultrastructural changes may be relevant. The epidemiology of microalbuminuria reveals a close association between systemic endothelial dysfunction and vascular disease, also implicating glomerular endothelial dysfunction in microalbuminuria. This review discusses the mechanisms of microalbuminuria in disease, particularly the emerging role of the glomerular endothelium and its glycocalyx, and examines its implications for cardiovascular disease in the pediatric population.

Introduction

The term 'microalbuminuria' is a relative misnomer: it implies 'small size' but actually refers to the presence of a relatively 'small quantity' of protein in the urine. The term was first used nearly 30 years ago when referring to urinary protein excretion of 30–300 mg per day, which was below the detection threshold of a standard urine dipstick test [1]. Microalbuminuria is now defined as a urine albumin excretion (UAE) between 20 and 200 µg/min or 30 to 300 mg in an overnight or 24-h collection. This range of UAE, although used in the pediatric population, is derived from population studies in adults.

Relevance of microalbuminuria

The importance of microalbuminuria as an independent predictor of progressive renal disease and cardiovascular mortality was thereafter realized in a number of prospective and epidemiological studies particularly in patients with diabetes [2–4] and hypertension [5]. In adults, the link between microalbuminuria, cardiovascular disease, and progressive renal disease is now well established in patients with systemic diseases including diabetes mellitus [6]. Interestingly, microalbuminuria has also emerged to be an important risk factor for the development of cardiovascular disease, and all cause mortality in the general population [7]. Faced with the realization of increasing prevalence of obesity, type 2 diabetes [8], and metabolic syndrome [9] in

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children, screening for microalbuminuria seems highly relevant in the pediatric population to detect and prevent cardiovascular disease. In this review, we discuss the current understanding of the pathophysiological mechanism underlying the appearance of increased albumin in the urine. We will review the epidemiological studies in microalbuminuria published regarding children and adolescents and discuss whether the implications for the pediatric population should be regarded as profound as in those for adults. During the discussions, we hold with the established view that increased trans-glomerular passage of albumin is the major source of microalbuminuria [10].

Methodology used to estimate microalbuminuria

Clinical studies in adults and children have used 24-h, overnight urine collections and spot urine testing to estimate UAE. Timed urine collections are generally more cumbersome in the pediatric population compared to spot urine testing for estimation for UAE. Simple alternatives include estimation of urine albumin concentration or albumin creatinine ratio estimated from spot urine samples. An albumin-to-creatinine ratio >10 mg/g is diagnostic of microalbuminuria and is shown to be superior to urine albumin concentration and comparable to 24-h urine collections [11, 12]. In adults, the use of the albumin-to-creatinine ratio particularly in the general population has been validated in a number of epidemiological studies [13–15] whereas in children there is relative paucity of such studies [16]. Due to a wider range of variation in UAE in children even within the normal range, it is recommended that the test should be repeated three times annually in diabetic subjects [17]. The mean albumin-to-creatinine ratio in normal children>6 years of age seems to fall between 8 and 10 mg/g (males: 7.5 mg/g; females 9.6 mg/g) [18]. Since, UAE is affected by exercise [19] and time of the day [20], early morning urine sample for the albumin creatinine ratio provides a more sensitive estimate of microalbuminuria. UAE is estimated to be the lowest in children<6 years old, followed by an increase through the adolescent years and a peak at age 15–16 years [16, 18]. Female gender [21], Tanner stage 4–5 of puberty, height, and weight [22] are all factors associated with a higher albumin excretion rate in healthy children. Cross-sectional studies of healthy adolescents have also reported higher albumin excretion in children of African American descent [23].

Prevalence of microalbuminuria

Data published from the third National Health and Nutritional Examination Survey (NHANES) [24] reported the prevalence of microalbuminuria in a sample of 22,244 subjects aged 6 to >80 years to be 7.8% (6.1% in males and 9.7% in females) with progressively increasing prevalence in adults >40 years of age. In contrast to the trend in adults, microalbuminuria among 6–19 year-olds (15%) was noted to be almost twofold more prevalent than in 20–39 year-olds adults (7.3%). Also, 6–19-year-old females even had higher prevalence rates than their male counterparts and were comparable to 60–79-year-old women. A further cross-sectional study analyzing the NHANES data for 12–19-year-old adolescents [25] showed that despite the presence of cardiovascular risk factors, overweight teenagers had a lower prevalence rate of microalbuminuria compared to healthy controls. Although low, within the group of obese children with microalbuminuria, there appeared to be significant association with the presence of hypertension, insulin resistance, and diabetes. The reason for the lower prevalence of microalbuminuria in obese teenagers is uncertain but perhaps lower exercise levels in obese teenagers leads to less confounding influence by orthostatic proteinuria [26] . This is opposite to the findings in adults where a number of studies have shown a positive relationship of obesity with microalbuminuria [27, 28]. As expected, microalbuminuria is five-fold more prevalent in children with diabetes [24].

One obvious explanation for the apparent increase in prevalence of microalbuminuria in healthy adolescents is possible confounding influence of orthostatic proteinuria. This has been reported in 20% of adolescents males [29] and increases with teenage years but tends to settle spontaneously after 20 years of age [30]. Orthostatic proteinuria in isolation is a benign condition and is not associated with long-term risk of renal disease [31, 32] but with a potential to mask underlying primary kidney disease [33]. Imaging studies show entrapment of the left renal vein in the fork between the aorta and proximal superior mesenteric artery in most cases of postural proteinuria [34, 35]. This is known as the 'nutcracker phenomenon'. Partial obstruction of left renal vein in an upright position leads rise in glomerular trans-capillary hydraulic pressure difference and efferent arteriolar resistance leading increased UAE. These hemodynamic changes are partially mediated via the actions of angiotensin II [36]. It is therefore important that orthostatic proteinuria is considered during assessment of children and adolescents with microalbuminuria.

Relationship between microalbuminuria and cardiovascular risk in children

In the adult population, there is indeed mounting evidence indicating the relationship between microalbuminuria and

cardiovascular risk [37]. The threshold level, however, to define normality in epidemiological studies is inconsistent. Post-hoc analyses of major clinical trials indicate that an incremental increase in microalbuminuria, even within the normal range is associated with an increased rate of cardiovascular events in adults [3, 5, 38]. This was also confirmed in a recent, meta-analysis of 105,872 subjects, which showed that the hazard ratio for all-cause and cardiovascular mortality (adjusted for age, genetics, history of CV disease, systolic blood pressure, diabetes, smoking, and total cholesterol) rises progressively with increase in UAE well below the microalbuminuria range, increasing to 1.83 with microalbuminuria in subjects with normal renal function [39].

The relationship between microalbuminuria and cardiovascular disease in the pediatric population is not as well studied as in adults [18]. There is a clear association between childhood obesity, elevated blood pressure, and high fasting insulin levels that persists into adulthood [9]. Childhood obesity, high blood pressure, and hyperlipidemia are also risk factors for developing atherosclerosis in young individuals [40]. It is well known that the pre-clinical atherosclerotic process beginning in childhood has the potential to be reversed [41]. Microalbuminuria seems to predict glucose intolerance and metabolic syndrome among obese children [42, 43]. Data from the Oxford Regional Prospective Study (ORPS), a population-based study of children with type 1 diabetes, has shown a significantly higher (50% compared to 34%) prevalence of microalbuminuria in children compared to adults corrected for glycemic control and duration of diabetes [44]. In their longitudinal study, Rademacher and colleagues [45] reported that adolescents with type 1 diabetes have higher UAE compared to normal healthy controls even prior to developing microalbuminuria. Body mass index, systolic blood pressure, and glycemic control predicted the onset of microalbuminuria and its progression was related to the duration of diabetes. In their healthy cohort, UAE was not correlated with an adverse outcome of blood pressure or insulin resistance [45]. In children with type 1 diabetes, endothelial dysfunction is known to coincide with microalbuminuria [46]. Exposure to hyperglycemia in unrecognized type 2 diabetes is also known to cause vascular complications earlier, with significantly higher rates of microalbuminuria at presentation [47, 48]. Interestingly, maternal blood pressure has been shown to predict microalbuminuria in young offspring with diabetes [49]. Type 2 diabetes diagnosed in early youth is associated with worse outcomes leading to higher rates of diabetic nephropathy and death, compared to adult-onset type 2 diabetes or non-diabetics [50]. Microalbuminuria is also a risk factor for development of left ventricular hypertrophy in adolescents with essential hypertension [51].

Overall, the evidence linking microalbuminuria and cardiovascular disease in healthy children is less convincing than in the adult population. Further studies with long-term follow-up of healthy children with microalbuminuria are needed. In obese children, microalbuminuria seems to be a consistent predictor of insulin resistance and hypertension, both of which are strong risks for future cardiovascular disease and death [52]. Again, there are no long-term studies with follow-up into late adult life to provide direct estimates of cardiovascular mortality. In children with diabetes, progression of UAE, (even within the normal range) starts early and is associated with worse cardiovascular outcomes than adults.

Microalbuminuria as a marker of generalized endothelial damage

The vascular endothelium, owing to its strategic location at the interface between flowing blood and other components of vascular wall, is sensitive to mechanical stimuli like shear stress and hormonal stimuli, from vasoactive substances. In response to these varied stimuli, it releases agents that regulate vasomotor function and inflammatory processes, and affect homeostasis. Vasodilator substances produced by the endothelium include nitric oxide (NO), prostacyclin, and C-type natriuretic peptide, which balance the vasoconstricting effect of endothelin-1, angiotensin II , thromboxane A2, and reactive oxygen species (ROS) [53]. Endothelial dysfunction is a well-known contributor to the pathophysiology of cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure [54]. Specific damage of the glomerular endothelial has been implicated in diseases like hemolytic uremic syndrome, pre-eclampsia, and acute ischemic renal injury [55–57].

Alteration in endothelial function is known to precede the development of morphological atherosclerotic changes and has a primary role in the development of a lesion and later clinical complications [58]. This process starts early in childhood [59, 60]. Markers of endothelial dysfunction like increased capillary permeability are known to be present well before the onset of microalbuminuria in type 1 diabetes and also show evidence of progression in association with it [61, 62]. This is difficult to establish in type 2 diabetes as it is often complicated by the presence of other risk factors for vascular disease at presentation and discerning the relationship of hyperglycemia and its sequelae to endothelial dysfunction is difficult. Although microalbuminuria may occur without the evidence of endothelial dysfunction, von Willebrand Factor levels, a marker of endothelial dysfunction, can predict its develop-

ment [63]. The link between endothelial dysfunction and microalbuminuria in type 1 diabetes seems to be important in predicting the development of diabetic nephropathy and susceptibility to micro- and macrovascular disease.

Endothelial dysfunction, as an important antecedent of microalbuminuria in both types of diabetes, provides an attractive explanation for the association between micro-albuminuria and vascular disease in diabetes, but is endothelial dysfunction enough to cause a breach in the sieving action of kidney leading to microalbuminuria? Glomerular endothelium is exposed to the same diabetic microenvironment as other endothelia, and it is highly likely that as a result of this exposure, it also becomes dysfunctional. Whether dysfunction of the glomerular endothelium can lead to microalbuminuria will be better understood once we consider the structure and function of the glomerular filtration barrier (GFB) in the next section.

Glomerular filtration barrier as a complex sieve

Glomerular capillaries are highly specialized and have high permeability to water (hydraulic conductivity) and small solutes yet, are highly resistant to the escape of macro-molecules (like albumin) in urine [64]. These properties are as a result of a unique, three-layer structure (Fig. 1) known as the glomerular filtration barrier (GFB). The GFB on the luminal side of the capillary is comprised of fenestrated glomerular endothelium with its glycocalyx, podocytes (glomerular visceral epithelial cells) on urinary side and glomerular basement membrane (GBM), which is sandwiched between the two cell types [65]. The GFB is primarily responsible for the sieving action of the kidney and in the healthy filters approximately 80 l of plasma every day.

Podocytes, or more specifically their inter-digitating foot processes, form the outer layer of the GFB (Fig. 1). The gaps between adjacent foot processes, known as the 'filtration slits' (25–60 nm), are spanned by the slit diaphragm. This is like a molecular scaffold thought to form the most restrictive barrier to water and macromolecular passage [66]. The effect of mutations in podocyte-specific proteins, which form the complex slit diaphragm (e.g., nephrin, mutations result in congenital nephrotic syndrome), indicate the importance of podocytes in restricting the passage of protein [67]. Alterations in
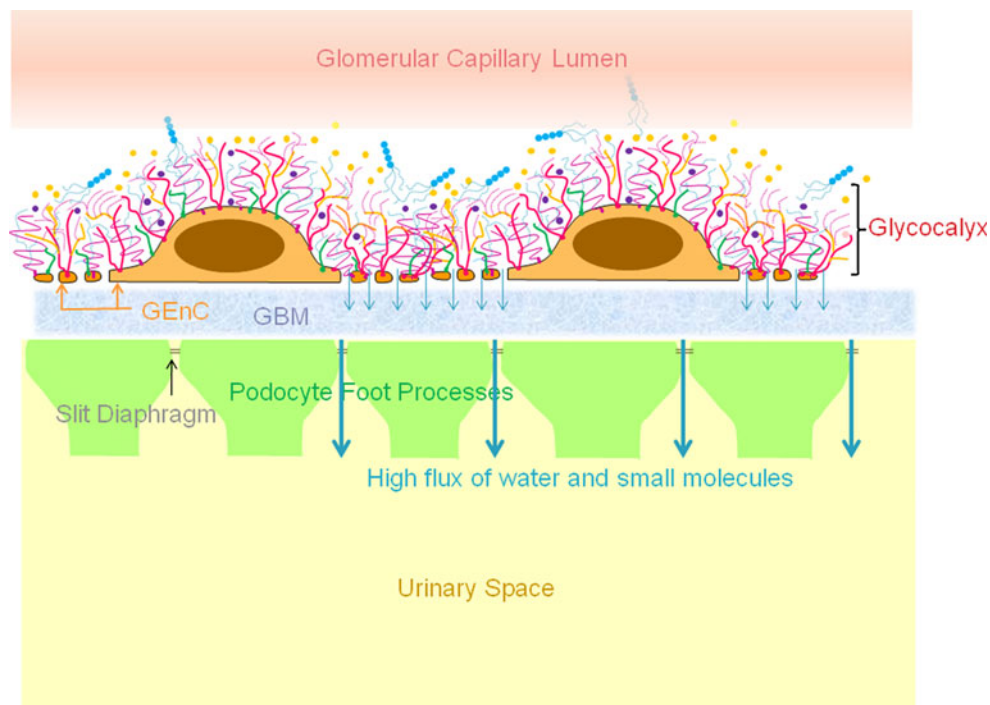

Fig. 1 Schematic drawing of components of the glomerular filtration barrier (GFB). Fenestrated glomerular endothelial cells (GEnC) form the luminal side of the sieve and facilitate the high flux of water and small molecules (*blue arrows*); glomerular basement membrane (GBM) in the middle, and the podocyte foot processes and slit diaphragms on the urinary side. The GEnC (including the fenestrae) are covered by a mesh-like, anionic layer of glycocalyx composed of sialic acid-rich glycoproteins and proteoglycans consisting of core proteins and attached branching glycosaminoglycan chains (mainly heparan sulphate and chondroitin sulphate). The glycosaminoglycan hyaluronan is non-covalently bound to the cell surface and other glycocalyx components. Adsorbed plasma proteins including albumin (*yellow dots*) and orosomucoid (*purple dots*) contribute to the high negative charge of the glycocalyx layer. In the healthy, all the three components of the GFB work together and conserve 99.9% of proteins in the capillary lumen

podocyte ultrastructure, particularly effacement of its foot process, are a common histological abnormality seen in kidney biopsy specimens and is associated with proteinuric kidney diseases including diabetes. Although discrete effacement of the podocyte foot process is an indicator of podocyte injury, it is an unreliable predictor of mild protein excretion [68–70].

The glomerular basement membrane (GBM) is a basal lamina specialized for the structural requirements of the GBM and its filtration function. It is a hydrated meshwork of collagens and laminins to which are attached negatively charged proteoglycans. Traditional concepts have therefore characterized the GBM as a charge-selective barrier. However, more recent analyses indicate that its contribution to the barrier to protein passage is small [65].

Glomerular endothelial cells are adapted to enable the high hydraulic conductivity of the GFB, necessary for filtration. These highly specialized cells have areas of numerous circular transcellular pores 60–80 nm in diameter [65, 71, 72] referred to as fenestrae. Initially these fenestrations were thought of as 'empty' and therefore providing little resistance to the passage of proteins. Advances in fixation techniques in electron microscopy have allowed the demonstration of glomerular endothelial glycocalyx which is 200–400 nm in thickness [73]. The glycocalyx covers both fenestral and inter-fenestral domains of the glomerular endothelial cell luminal surface at the interface of the endothelium and the blood flow [74].

The glycocalyx is a dynamic hydrated layer largely composed of glycoproteins and proteoglycans with adsorbed plasma proteins. Proteoglycans, particularly heparan sulphate proteoglycans (HSPG), are largely responsible for the anionic charge characteristics of the glycocalyx. Selective removal of endothelial glycocalyx from coronary vessels increases permeability providing evidence that it constitutes a barrier to macromolecular permeability [75].

Therefore, the presence of a significant glomerular endothelial glycocalyx implies that the glomerular endothelium contributes significantly to the barrier to macromolecules [76, 77]. Experimental data certainly supports this view. Mice treated with glycocalyx-degrading enzymes show reduction in thickness of glomerular endothelial glycocalyx coinciding with increased albumin excretion [78]. In rats, under physiological perfusion conditions, albumin is confined to the glomerular capillary lumen and endothelial fenestrae, implying resistance at the level of glomerular endothelial surface [79]. Endothelial glycocalyx has the correct anatomical distribution (on the surface of endothelial cells including in fenestral openings) to explain this distribution. Reactive oxygen species (ROS), which are known to damage the glycocalyx [80], cause heavy proteinuria without any identifiable structural changes in the GFB using standard electron microscopy techniques

[81]. Interestingly, a recent study showed that disruption of glomerular endothelial glycocalyx by induction of ROS can cause proteinuria [82]. Furthermore the ability of human glomerular endothelial cell glycocalyx to form a permeability barrier to macromolecules can also be directly demonstrated in vitro [77].

Structural and functional alterations in GFB associated with microalbuminuria

Even though the glomerular structural changes typical of glomerular diseases like diabetic nephropathy are commonly established by the time microalbuminuria becomes apparent [83], these changes are heterogeneous and can be found in patients without microalbuminuria [70]. Early changes include an increase in glomerular size, thickening of the GBM, expansion of the mesangium, and effacement of podocyte foot processes [70, 83]. The increase in glomerular size is due both to mesangial expansion and to enlargement in glomerular capillaries due to hemodynamic changes. Glomerular structural changes are less marked in type 2 diabetes, with only a third conforming to the classical pattern observed in type 1 diabetes [84].

Effacement of podocyte foot processes is an indicator of podocyte injury, but an unreliable predictor of protein excretion. Surely, proteinuria can occur in complete absence of structural changes in podocytes [85]. This is particularly evident in diabetic microalbuminuria associated with type 2 diabetes where podocyte ultrastructure may be unchanged [86]. Perhaps a likely explanation to reconcile this issue is that the lower proportion of podocytes seen in early disease is due to an increase in mesangial and endothelial cells while podocyte loss occurs at a later stage.

As discussed in the previous section, the hypothesis brought forward is that glomerular endothelial glycocalyx is well placed to play an important role in the pathogenesis of microalbuminuria. There is emerging evidence showing that total systemic glycocalyx volume is reduced by acute hyperglycemia in human subjects [87]. Also, type 1 diabetics have reduced systemic glycocalyx volume, which coincides with the onset of microalbuminuria [88]. GBM thickening alone, without change in composition, does not significantly affect its protein permeability characteristics.

Increased passage of albumin across the GFB in diabetic microalbuminuria can be confirmed in experimental models. Detailed analysis of permeability characteristics of the GFB to molecules of varying size and charge has been used to estimate whether the increased flux of albumin is due to loss of size or charge selectivity of the GFB. In the animal models of diabetes the primary defect seems to be in charge selectivity [89], but in healthy non-diabetic individuals with microalbuminuria, loss of both charge and size selectivity

of the GFB is seen [90]. In both type 1 and type 2 diabetes, defects in charge-selectivity occur earlier than loss of size selectivity [86, 91].

Conclusions

In summary, microalbuminuria in the healthy pediatric population appears to be more prevalent than in young adults. Obesity is an established risk factor for diabetes and metabolic syndrome. However, obese children in the absence of diabetes do not have a higher prevalence of microalbuminuria. Microalbuminuria in non-diabetic and non-obese children is not associated with cardiovascular risk factors. In contrast, in obese children, cardiovascular risk factors such as impaired fasting glucose, insulin resistance, and hypertension are strongly associated with microalbuminuria. This shows that the association between cardiovascular risk factors and microalbuminuria is strongly modified by being overweight.

Unlike the literature in adults, there is a paucity of clinical evidence from longitudinal studies that analyzes the long-term cardiovascular risks in healthy children with microalbuminuria. In diabetic children, the onset and progression of micro-albuminuria carries a similar prognostic profile to adults. Microalbuminuria is an indicator of generalized endothelial dysfunction and is regarded as a common pathway of injury to both renal and systemic vascular beds. Progression of micro-albuminuria to overt nephropathy is accompanied by predict-able structural changes in the glomerulus including loss and damage to the podocyte. The endothelial glycocalyx, apart from acting as a barrier to protein permeability, also protects the endothelium against atherosclerosis [92]. Disturbance of the endothelial glycocalyx as the common process underly-ing both microalbuminuria and generalized endothelial dysfunction is the most plausible explanation for the profound cardiovascular complications seen in these patients.

The evidence from studies in the pediatric population does not provide clear estimates of cardiovascular risks in relatively healthy children who develop microalbumi-nuria and clearly further epidemiological studies are necessary to determine its ramifications. Therefore, currently widespread screening in the pediatric popula-tion cannot be justified. However, studies so far provide enough evidence to suggest that microalbuminuria in obese children, even in the absence of diabetes, should be taken as seriously as in diabetic children. We recommend that overweight children and parents should be motivated early on and engaged for lifestyle advice (exercise, cessation of smoking, diet) with close moni-toring. Evidence from small studies suggests that early intervention with ACE inhibitor therapy is likely to be

beneficial [93, 94]. The Adolescent type 1 Diabetes cardio-renal Intervention Trial (AdDIT) is an ongoing multi-center, randomized, double-blind clinical trial that will hopefully report on the use of ACE inhibitor and statin therapy in adolescents with type 1 diabetes and hopefully provide long-term data on cardiovascular out-comes after this intervention [95]. Clinicians should also give careful consideration to progression of UAE rather than being rigid about the threshold for microalbuminuria.

Finally, we hope that with increasing understanding of the molecular mechanisms of microalbuminuria, novel therapies will be designed that prevent and reverse endothelial dysfunction association with microalbuminuria and the resultant cardiovascular disease.

Multiple-choice questions (answers appear following the reference list):

1. In estimating microalbuminuria, the following statement is true:
   a) early morning spot urine for albumin creatinine ratio is the best
   b) 24-h urine collection should always be done when possible
   c) urine protein creatinine ratio is as reliable as the urine albumin creatinine ratio
   d) the standard urine dip stick in the clinic is equally reliable
2. The estimates of prevalence of microalbuminuria in children are:
   a) higher compared to young adults
   b) higher in girls and children from an ethnic background
   c) are likely confounded by the effects of orthostatic proteinuria
   d) all of the above
3. Postural or orthostatic proteinuria
   a) is not a benign condition and indicates progressive kidney disease
   b) more common in obese girls
   c) mostly associated with 'nutcracker syndrome'
   d) should be managed with ACE inhibitors
4. Generalized endothelial dysfunction is
   a) rarely seen in children
   b) usually precedes the onset of microalbuminuria
   c) is reversed in early adult life
   d) is exclusively seen in diabetic children
5. The glomerular endothelial glycocalyx layer
   a) is a dynamic layer that is produced by glomerular endothelial cells
   b) is vulnerable to damage in systemic diseases
   c) is an important contributor to resistance to protein permeability
   d) all of the above

References

1. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H (1982) Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1:1430–1432

2. Dinneen SF, Gerstein HC (1997) The association of micro-albuminuria and mortality in non-insulin-dependent diabetes mellitus. A systematic overview of the literature. Arch Intern Med 157:1413–1418

3. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Halle JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S (2001) Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA 286:421–426

4. Allen KV, Walker JD (2003) Microalbuminuria and mortality in long-duration type 1 diabetes. Diab Care 26:2389–2391

5. Wachtell K, Ibsen H, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlof B, Devereux RB, Beevers G, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Kristianson K, Lederballe-Pedersen O, Nieminen MS, Okin PM, Omvik P, Oparil S, Wedel H, Snapinn SM, Aurup P (2003) Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. Ann Intern Med 139:901–906

6. Strippoli GF, Craig M, Deeks JJ, Schena FP, Craig JC (2004) Effects of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists on mortality and renal outcomes in diabetic nephropathy: systematic review. BMJ 329:828

7. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE (2002) Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. Circulation 106:1777–1782

8. Rosenbloom AL, Joe JR, Young RS, Winter WE (1999) Emerging epidemic of type 2 diabetes in youth. Diab Care 22:345–354

9. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S (2004) Obesity and the metabolic syndrome in children and adolescents. N Engl J Med 350:2362–2374

10. Haraldsson B, Nystrom J, Deen WM (2008) Properties of the glomerular barrier and mechanisms of proteinuria. Physiol Rev 88:451–487

11. Dyer AR, Greenland P, Elliott P, Daviglus ML, Claeys G, Kesteloot H, Ueshima H, Stamler J (2004) Evaluation of measures of urinary albumin excretion in epidemiologic studies. Am J Epidemiol 160:1122–1131

12. Bakker AJ (1999) Detection of microalbuminuria. Receiver operating characteristic curve analysis favors albumin-to-creatinine ratio over albumin concentration. Diab Care 22:307–313

13. Jafar TH, Chaturvedi N, Hatcher J, Levey AS (2007) Use of albumin creatinine ratio and urine albumin concentration as a screening test for albuminuria in an Indo-Asian population. Nephrol Dial Transplant 22:2194–2200

14. Gansevoort RT, Brinkman J, Bakker SJ, De Jong PE, de Zeeuw D (2006) Evaluation of measures of urinary albumin excretion. Am J Epidemiol 164:725–727

15. Brantsma AH, Atthobari J, Bakker SJ, de Zeeuw D, de Jong PE, Gansevoort RT (2007) What predicts progression and regression of urinary albumin excretion in the nondiabetic population? J Am Soc Nephrol 18:637–645

16. Sanchez-Bayle M, Rodriguez-Cimadevilla C, Asensio C, Ruiz-Jarabo C, Baena J, Arnaiz P, Villa S, Cocho P (1995) Urinary albumin excretion in Spanish children. Nino Jesus Group. Pediatr Nephrol 9:428–430

17. Gibb DM, Shah V, Preece M, Barratt TM (1989) Variability of urine albumin excretion in normal and diabetic children. Pediatr Nephrol 3:414–419

18. Rademacher ER, Sinaiko AR (2009) Albuminuria in children. Curr Opin Nephrol Hypertens 18:246–251

19. Jefferson IG, Greene SA, Smith MA, Smith RF, Griffin NK, Baum JD (1985) Urine albumin to creatinine ratio-response to exercise in diabetes. Arch Dis Child 60:305–310

20. Marshall SM (1991) Screening for microalbuminuria: which measurement? Diabet Med 8:706–711

21. Davies AG, Postlethwaite RJ, Price DA, Burn JL, Houlton CA, Fielding BA (1984) Urinary albumin excretion in school children. Arch Dis Child 59:625–630

22. Skinner AM, Addison GM, Price DA (1996) Changes in the urinary excretion of creatinine, albumin and N-acetyl-beta-D-glucosaminidase with increasing age and maturity in healthy schoolchildren. Eur J Pediatr 155:596–602

23. Hanevold CD, Pollock JS, Harshfield GA (2008) Racial differences in microalbumin excretion in healthy adolescents. Hypertension 51:334–338

24. Jones CA, Francis ME, Eberhardt MS, Chavers B, Coresh J, Engelgau M, Kusek JW, Byrd-Holt D, Narayan KM, Herman WH, Jones CP, Salive M, Agodoa LY (2002) Microalbuminuria in the US population: third National Health and Nutrition Examination Survey. Am J Kidney Dis 39:445–459

25. Nguyen S, McCulloch C, Brakeman P, Portale A, Hsu CY (2008) Being overweight modifies the association between cardiovascular risk factors and microalbuminuria in adolescents. Pediatrics 121:37–45

26. Janssen I, Katzmarzyk PT, Boyce WF, Vereecken C, Mulvihill C, Roberts C, Currie C, Pickett W (2005) Comparison of overweight and obesity prevalence in school-aged youth from 34 countries and their relationships with physical activity and dietary patterns. Obes Rev 6:123–132

27. Bahrami H, Bluemke DA, Kronmal R, Bertoni AG, Lloyd-Jones DM, Shahar E, Szklo M, Lima JA (2008) Novel metabolic risk factors for incident heart failure and their relationship with obesity: the MESA (Multi-Ethnic Study of Atherosclerosis) study. J Am Coll Cardiol 51:1775–1783

28. Sharma K (2009) The link between obesity and albuminuria: adiponectin and podocyte dysfunction. Kidney Int 76:145–148

29. Brandt JR, Jacobs A, Raissy HH, Kelly FM, Staples AO, Kaufman E, Wong CS (2010) Orthostatic proteinuria and the spectrum of diurnal variability of urinary protein excretion in healthy children. Pediatr Nephrol 25:1131–1137

30. Springberg PD, Garrett LE Jr, Thompson AL Jr, Collins NF, Lordon RE, Robinson RR (1982) Fixed and reproducible orthostatic protein-uria: results of a 20-year follow-up study. Ann Intern Med 97:516–519

31. Robinson RR (1980) Isolated proteinuria in asymptomatic patients. Kidney Int 18:395–406

32. Rytand DA, Spreiter S (1981) Prognosis in postural (orthostatic) proteinuria: forty to fifty-year follow-up of six patients after diagnosis by Thomas Addis. N Engl J Med 305:618–621

33. Berns JS, McDonald B, Gaudio KM, Siegel NJ (1986) Progression of orthostatic proteinuria to focal and segmental glomerulo-sclerosis. Clin Pediatr Phila 25:165–166

34. Ragazzi M, Milani G, Edefonti A, Burdick L, Bianchetti MG, Fossali EF (2008) Left renal vein entrapment: a frequent feature in children with postural proteinuria. Pediatr Nephrol 23:1837–1839

35. Milani GP, Mazzoni MB, Burdick L, Bianchetti MG, Fossali EF (2010) Postural proteinuria associated with left renal vein entrapment: a follow-up evaluation. Am J Kidney Dis 55:e29–e31

36. Yoshioka T, Mitarai T, Kon V, Deen WM, Rennke HG, Ichikawa I (1986) Role for angiotensin II in an overt functional proteinuria. Kidney Int 30:538–545

37. Ritz E (2006) Heart and kidney: fatal twins? Am J Med 119:S31–S39

38. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, Jensen G, Clausen P, Scharling H, Appleyard M, Jensen JS (2004) Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. Circulation 110:32–35

39. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, Coresh J, Gansevoort RT (2010) Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet 375:2073–2081

40. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA (1998) Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 338:1650–1656

41. Charakida M, Deanfield JE, Halcox JP (2007) Childhood origins of arterial disease. Curr Opin Pediatr 19:538–545

42. Burgert TS, Dziura J, Yeckel C, Taksali SE, Weiss R, Tamborlane W, Caprio S (2006) Microalbuminuria in pediatric obesity: prevalence and relation to other cardiovascular risk factors. Int J Obes Lond 30:273–280

43. Invitti C, Maffeis C, Gilardini L, Pontiggia B, Mazzilli G, Girola A, Sartorio A, Morabito F, Viberti GC (2006) Metabolic syndrome in obese Caucasian children: prevalence using WHO-derived criteria and association with nontraditional cardiovascular risk factors. Int J Obes Lond 30:627–633

44. Amin R, Widmer B, Prevost AT, Schwarze P, Cooper J, Edge J, Marcovecchio L, Neil A, Dalton RN, Dunger DB (2008) Risk of microalbuminuria and progression to macroalbuminuria in a cohort with childhood onset type 1 diabetes: prospective observational study. BMJ 336:697–701

45. Rademacher E, Mauer M, Jacobs DR Jr, Chavers B, Steinke J, Sinaiko A (2008) Albumin excretion rate in normal adolescents: relation to insulin resistance and cardiovascular risk factors and comparisons to type 1 diabetes mellitus patients. Clin J Am Soc Nephrol 3:998–1005

46. Ladeia AM, Ladeia-Frota C, Pinho L, Stefanelli E, Adan L (2005) Endothelial dysfunction is correlated with microalbuminuria in children with short-duration type 1 diabetes. Diab Care 28:2048–2050

47. Haffner SM, Gonzales C, Valdez RA, Mykkanen L, Hazuda HP, Mitchell BD, Monterrosa A, Stern MP (1993) Is microalbuminuria part of the prediabetic state? The Mexico City Diabetes Study. Diabetologia 36:1002–1006

48. Eppens MC, Craig ME, Cusumano J, Hing S, Chan AK, Howard NJ, Silink M, Donaghue KC (2006) Prevalence of diabetes complications in adolescents with type 2 compared with type 1 diabetes. Diab Care 29:1300–1306

49. Marcovecchio ML, Tossavainen PH, Acerini CL, Barrett TG, Edge J, Neil A, Shield J, Widmer B, Dalton RN, Dunger DB (2010) Maternal but not paternal association of ambulatory blood pressure with albumin excretion in young offspring with type 1 diabetes. Diab Care 33:366–371

50. Pavkov ME, Bennett PH, Knowler WC, Krakoff J, Sievers ML, Nelson RG (2006) Effect of youth-onset type 2 diabetes mellitus on incidence of end-stage renal disease and mortality in young and middle-aged Pima Indians. JAMA 296:421–426

51. Assadi F (2008) Relation of left ventricular hypertrophy to microalbuminuria and C-reactive protein in children and adolescents with essential hypertension. Pediatr Cardiol 29:580–584

52. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC (2010) Childhood obesity, other cardiovascular risk factors, and premature death. N Engl J Med 362:485–493

53. Schiffrin EL (2001) A critical review of the role of endothelial factors in the pathogenesis of hypertension. J Cardiovasc Pharmacol 38(Suppl 2):S3–S6

54. Endemann DH, Schiffrin EL (2004) Endothelial dysfunction. J Am Soc Nephrol 15:1983–1992

55. Sutton TA, Fisher CJ, Molitoris BA (2002) Microvascular endothelial injury and dysfunction during ischemic acute renal failure. Kidney Int 62:1539–1549

56. Davison JM, Homuth V, Jeyabalan A, Conrad KP, Karumanchi SA, Quaggin S, Dechend R, Luft FC (2004) New aspects in the pathophysiology of preeclampsia. J Am Soc Nephrol 15:2440–2448

57. van Setten PA, van Hinsbergh VW, van der Velden TJ, van de Kar NC, Vermeer M, Mahan JD, Assmann KJ, van den Heuvel LP, Monnens LA (1997) Effects of TNF alpha on verocytotoxin cytotoxicity in purified human glomerular microvascular endothelial cells. Kidney Int 51:1245–1256

58. Deanfield JE, Halcox JP, Rabelink TJ (2007) Endothelial function and dysfunction: testing and clinical relevance. Circulation 115:1285–1295

59. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE (1994) Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol 24:471–476

60. Halcox JP, Deanfield JE (2005) Childhood origins of endothelial dysfunction. Heart 91:1272–1274

61. Schalkwijk CG, Poland DC, van Dijk W, Kok A, Emeis JJ, Drager AM, Doni A, van Hinsbergh VW, Stehouwer CD (1999) Plasma concentration of C-reactive protein is increased in type I diabetic patients without clinical macroangiopathy and correlates with markers of endothelial dysfunction: evidence for chronic inflammation. Diabetologia 42:351–357

62. Stehouwer CD, Fischer HR, van Kuijk AW, Polak BC, Donker AJ (1995) Endothelial dysfunction precedes development of microalbuminuria in IDDM. Diabetes 44:561–564

63. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH (2002) Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes 51:1157–1165

64. Deen WM, Lazzara MJ (2004) Glomerular filtration of albumin: how small is the sieving coefficient? Kidney Int Suppl:S63-64.

65. Deen WM, Lazzara MJ, Myers BD (2001) Structural determinants of glomerular permeability. Am J Physiol Ren Physiol 281:F579–F596

66. Endlich K, Kriz W, Witzgall R (2001) Update in podocyte biology. Curr Opin Nephrol Hypertens 10:331–340

67. Pavenstadt H, Kriz W, Kretzler M (2003) Cell biology of the glomerular podocyte. Physiol Rev 83:253–307

68. Sharma SG, Spencer T, Gokden N (2010) The significance of foot process effacement in immunoglobulin a nephropathy: clinico-pathologic study of 161 cases with light, immunofluorescence and electron microscopic studies. Ultrastruct Pathol 34:279–272

69. Deegens JK, Dijkman HB, Borm GF, Steenbergen EJ, van den Berg JG, Weening JJ, Wetzels JF (2008) Podocyte foot process effacement as a diagnostic tool in focal segmental glomerulosclerosis. Kidney Int 74:1568–1576

70. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, Coplon NS, Sun L, Meyer TW (1997) Podocyte loss and progressive glomerular injury in type II diabetes. J Clin Invest 99:342–348

71. Ballermann BJ (2007) Contribution of the endothelium to the glomerular permselectivity barrier in health and disease. Nephron Physiol 106:p19–p25

72. Satchell SC, Braet F (2009) Glomerular endothelial cell fenestrations: an integral component of the glomerular filtration barrier. Am J Physiol Ren Physiol 296:F947–F956

73. Hjalmarsson C, Johansson BR, Haraldsson B (2004) Electron microscopic evaluation of the endothelial surface layer of glomerular capillaries. Microvasc Res 67:9–17

74. Rostgaard J, Qvortrup K (2002) Sieve plugs in fenestrae of glomerular capillaries–site of the filtration barrier? Cells Tissues Organs 170:132–138

75. Huxley VH, Williams DA (2000) Role of a glycocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments. Am J Physiol Heart Circ Physiol 278:H1177–H1185

76. Ballermann BJ, Stan RV (2007) Resolved: capillary endothelium is a major contributor to the glomerular filtration barrier. J Am Soc Nephrol 18:2432–2438

77. Singh A, Satchell SC, Neal CR, McKenzie EA, Tooke JE, Mathieson PW (2007) Glomerular endothelial glycocalyx constitutes a barrier to protein permeability. J Am Soc Nephrol 18:2885–2893

78. Jeansson M, Haraldsson B (2006) Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. Am J Physiol Ren Physiol 290:F111–F116

79. Ryan GB, Karnovsky MJ (1976) Distribution of endogenous albumin in the rat glomerulus: role of hemodynamic factors in glomerular barrier function. Kidney Int 9:36–45

80. Henry CB, Duling BR (2000) TNF-alpha increases entry of macromolecules into luminal endothelial cell glycocalyx. Am J Physiol Heart Circ Physiol 279:H2815–H2823

81. Yoshioka T, Ichikawa I, Fogo A (1991) Reactive oxygen metabolites cause massive, reversible proteinuria and glomerular sieving defect without apparent ultrastructural abnormality. J Am Soc Nephrol 2:902–912

82. Kuwabara A, Satoh M, Tomita N, Sasaki T, Kashihara N (2010) Deterioration of glomerular endothelial surface layer induced by oxidative stress is implicated in altered permeability of macromolecules in Zucker fatty rats. Diabetologia 53:2056–2065

83. Dalla Vestra M, Saller A, Bortoloso E, Mauer M, Fioretto P (2000) Structural involvement in type 1 and type 2 diabetic nephropathy. Diab Metab 26(Suppl 4):8–14

84. Fioretto P, Stehouwer CD, Mauer M, Chiesura-Corona M, Brocco E, Carraro A, Bortoloso E, van Hinsbergh VW, Crepaldi G, Nosadini R (1998) Heterogeneous nature of microalbuminuria in NIDDM: studies of endothelial function and renal structure. Diabetologia 41:233–236

85. Karumanchi SA, Epstein FH, Stillman IE (2005) Is loss of podocyte foot processes necessary for the induction of proteinuria? Am J Kidney Dis 45:436

86. Lemley KV, Blouch K, Abdullah I, Boothroyd DB, Bennett PH, Myers BD, Nelson RG (2000) Glomerular permselectivity at the onset of nephropathy in type 2 diabetes mellitus. J Am Soc Nephrol 11:2095–2105

87. Nieuwdorp M, van Haeften TW, Gouverneur MC, Mooij HL, van Lieshout MH, Levi M, Meijers JC, Holleman F, Hoekstra JB, Vink H, Kastelein JJ, Stroes ES (2006) Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes 55:480–486

88. Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JA, Ince C, Holleman F, Diamant M, Heine RJ, Hoekstra JB, Kastelein JJ, Stroes ES, Vink H (2006) Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. Diabetes 55:1127–1132

89. Jeansson M, Granqvist AB, Nystrom JS, Haraldsson B (2006) Functional and molecular alterations of the glomerular barrier in long-term diabetes in mice. Diabetologia 49:2200–2209

90. Jensen JS, Borch-Johnsen K, Deckert T, Deckert M, Jensen G, Feldt-Rasmussen B (1995) Reduced glomerular size- and charge-selectivity in clinically healthy individuals with microalbuminuria. Eur J Clin Invest 25:608–614

91. Deckert T, Kofoed-Enevoldsen A, Vidal P, Norgaard K, Andreasen HB, Feldt-Rasmussen B (1993) Size- and charge selectivity of glomerular filtration in Type 1 (insulin-dependent) diabetic patients with and without albuminuria. Diabetologia 36:244–251

92. Noble MI, Drake-Holland AJ, Vink H (2008) Hypothesis: arterial glycocalyx dysfunction is the first step in the atherothrombotic process. QJM 101:513–518

93. Cook J, Daneman D, Spino M, Sochett E, Perlman K, Balfe JW (1990) Angiotensin converting enzyme inhibitor therapy to decrease microalbuminuria in normotensive children with insulin-dependent diabetes mellitus. J Pediatr 117:39–45

94. Rudberg S, Aperia A, Freyschuss U, Persson B (1990) Enalapril reduces microalbuminuria in young normotensive type 1 (insulin-dependent) diabetic patients irrespective of its hypotensive effect. Diabetologia 33:470–476

95. (2009) Adolescent type 1 Diabetes Cardio-renal Intervention Trial (AdDIT). BMC Pediatr 9:79.

Answers:
1. a
2. d
3. c
4. b
5. d