
Microalbuminuria and Endothelial Dysfunction: Emerging Targets for Primary Prevention of End-organ Damage

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Abstract: A minor increase in urinary albumin excretion (microalbuminuria) is known to predict adverse renal and cardiovascular events in diabetic and hypertensive patients. Recent intriguing findings show that microalbuminuria is an early and sensitive marker of future cardiovascular events even in healthy subjects. The mechanisms linking microalbuminuria with end-organ damage have not been fully explained yet; however, generalized endothelial dysfunction might play an important role. Prevailing experimental and clinical data suggest that generalized endothelial dysfunction, frequently characterized by decreased nitric oxide bioavailability, actually precedes the development of microalbuminuria. This review summarizes the current knowledge about the intricate relationship between microalbuminuria and endothelial dysfunction. On the basis of the current evidence, we propose that microalbuminuria and endothelial dysfunction are an emerging target for primary prevention strategies in cardiovascular disease. In near future, dietary components improving nitric oxide bioavailability, such as cocoa-derived flavanols may play important role in these preventive strategies.

Key Words: microalbuminuria, endothelial dysfunction, cardiovascular disease, primary prevention, predictive value, cocoa, flavanols

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End-organ damage associated with cardiovascular disease is the leading cause of morbidity and mortality in the Western world. Moreover, the costs related to the end-organ damage, such as chronic kidney or heart failure, constitute the majority of expenditures in the total health care budget.¹ Therefore, a shift is required from secondary prevention of renal and cardiac end-organ damage to primary prevention targeting the individuals with an increased risk profile at an early stage of the disease. Disclosure of novel markers for increased risk

may help to identify specific individuals and assist in tailoring prevention according to their individual risk profile.

In addition to traditional cardiovascular risk factors, such as hypertension, dyslipidemia, central obesity, hyperglycemia, and smoking, microalbuminuria has recently received a great deal of attention as a new, accessible, and sensitive marker of renal and cardiovascular risk.^{2–4} The mechanisms linking microalbuminuria to increased renal and cardiovascular risk are not fully understood, but it has been proposed that microalbuminuria is a reflection of generalized endothelial dysfunction.⁵ This review summarizes the current knowledge about the intricate relationship between microalbuminuria and endothelial dysfunction. It focuses on the role of these 2 parameters as early markers of both renal and cardiovascular disease. On the basis of the current evidence, we suggest that microalbuminuria and endothelial dysfunction represent emerging targets for primary prevention strategies. Because endothelial dysfunction is frequently characterized by decreased bioavailability of nitric oxide (NO), we propose that dietary components improving NO bioavailability, such as cocoa-derived flavanols, may play important role in these preventive strategies.

MICROALBUMINURIA

Definition

Albumin is the major constituent of proteins excreted in urine. The widely used dipstick method detects only albumin excretion exceeding 300 mg per 24 hours, a range currently defined as macroalbuminuria or proteinuria. However, studies in diabetic patients demonstrated that much lower values of albumin excretion are associated with increased risk for the development of diabetic nephropathy.^{6,7} Currently, the range between 30 and 300 mg per 24 hours, or 20 to 200 µg/min measured overnight is defined as microalbuminuria. Methods for the measurements of urinary albumin, its definition, and classification have been extensively summarized elsewhere.^{8,9}

Microalbuminuria is Not Rare in Normal Healthy Population

Prevalence of microalbuminuria has been initially studied in individuals with diabetes and later in

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hypertensive cohorts. Still, a large variability is reported in distinct clinical trials, probably because of heterogeneity of the study population, regarding age, race, severity of hypertension, coexistence of nephropathy, antihypertensive medication, and associated lipid abnormalities. Methods of detection and sampling techniques might form an additional source of variation among trials. In general, a prevalence of 20% to 40% in patients with diabetes mellitus is reported in large studies.^{10–13} In the individuals with essential hypertension the prevalence of microalbuminuria seems somewhat lower.^{14,15} As an example, microalbuminuria was found in 23% of patients with essential hypertension and left ventricular hypertrophy included in the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) study.¹⁶

Increased incidence of microalbuminuria is not exclusively limited to the populations with elevated cardiovascular risk. An increasing number of large trials suggest that microalbuminuria is also common in a general, “healthy” population. In one of the largest cohorts studying a general population, the Prevention of Renal and Vascular End stage Disease (PREVEND) study, a prevalence of 7.2% was reported in 40,856 subjects and 6.6% after exclusion of the subjects with diabetes mellitus and hypertension.¹⁷ Several other cross-sectional studies confirm 5% to 8% prevalence of microalbuminuria in the general population,^{18–20} suggesting that among healthy individuals a considerable variability in urinary albumin excretion already exists without the presence of any clinical condition. This phenomenon renders microalbuminuria a promising candidate as the integrated marker of cardiovascular risk in the general population.

Microalbuminuria Predicts Renal and Cardiovascular Outcome in Diseased and Healthy Population

Considerable attention for microalbuminuria as a predictive parameter stemmed from the publication of Viberti et al⁷ establishing the predictive value of microalbuminuria for nephropathy in insulin-dependent diabetes mellitus. Since then several studies have confirmed elevated albumin excretion as a marker for the development of diabetic nephropathy and progressive renal failure both in patients with type 1⁶ and type 2 diabetes.²¹ However, the predictive value of microalbuminuria in diabetics is not only limited to renal events, as microalbuminuria predicts total and especially cardiovascular mortality and morbidity in several studies in non-insulin-dependent diabetic populations²² even after adjustment for other conventional cardiovascular risk factors. Yudkin²³ were the first to report this association also in nondiabetic subjects. By now, it is well established that microalbuminuria identifies the individuals with adverse prognosis among hypertensive patients. In one of the largest longitudinal studies performed to investigate a predictive role of microalbuminuria, the Danish MONICA study, hypertensive subjects with albuminuria showed almost 4-fold increased risk of ischemic heart

disease as compared with normoalbuminuric hypertensive subjects.²⁴ In the prospective LIFE trial in nondiabetic hypertensive patients with left ventricular hypertrophy, levels of albumin excretion at entry were predictive for composite end point.²⁵ Because there was no threshold for the increased risk, correlation between albuminuria and risk exists also at albumin levels below the current definition of microalbuminuria.

Whereas microalbuminuria is clearly related to cardiovascular risk in high-risk populations, it is important to mention that its predictive value also extrapolates to the general population. In the previously mentioned prospective Danish MONICA study, microalbuminuric subjects in general population were at increased risk for ischemic heart disease.²⁶ Furthermore, Hillege et al¹⁷ demonstrated that microalbuminuria was independently associated with cardiovascular risk factors and morbidity in the general population, on the basis of cross-sectional analysis of the baseline data from the PREVEND study. In a more recent prospective analysis of this study, microalbuminuria independently predicted cardiovascular and all-cause mortality in the general population (Fig. 1).²⁷ Moreover, the relationship was already apparent at levels of albumin excretion considered to be normal. Within the same cohort, Verhave et al²⁸ showed that subjects with microalbuminuria at baseline had a higher chance to develop de novo impairment of renal function in a 4-year follow-up.

Taken together, these data demonstrate the usefulness of microalbuminuria as a valuable and clinically relevant tool for the identification of individual patients at risk for the development of end-organ damage, for example, renal and systemic cardiovascular disease. An important question is how such a relationship may be explained and what the link is between increased urinary excretion of albumin and end-organ damage. Currently, the evidence points towards the hypothesis that microalbuminuria is a reflection of *generalized systemic endothelial dysfunction*.

MICROALBUMINURIA AND ENDOTHELIAL DYSFUNCTION AS PROGNOSTIC MARKERS

The presence of elevated concentrations of albumin in the urine signifies impaired glomerular permeability for plasma proteins. The coincidence of microalbuminuria and extrarenal vascular damage in diabetic patients led to the hypothesis that albuminuria does not only reflect a state of glomerular, but also generalized endothelial dysfunction.⁵ Consequently, generalized endothelial dysfunction might explain the prognostic value of microalbuminuria in systemic cardiovascular events and end-organ damage. Although most of the observations have supported this hypothesis, some important questions remain unanswered. Two important issues are whether endothelial dysfunction actually precedes microalbuminuria and whether it might also be used as an early prognostic marker of end-organ damage in a healthy population. In the next section, we will summarize the

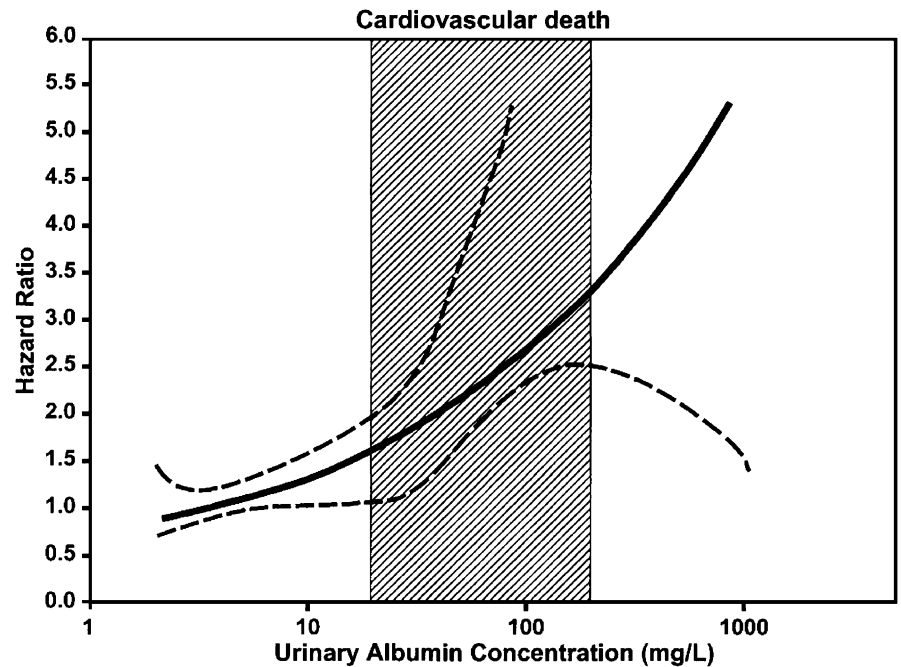


FIGURE 1. Urinary albumin excretion predicts cardiovascular mortality in the general population; Relationship between urinary albumin excretion and hazard ratio. The dotted lines represent 95% confidence limits and the squared area indicates the definition of microalbuminuria. Reproduced from Ref. 27.

concept of endothelial dysfunction, discuss the intricate relation between microalbuminuria and endothelial dysfunction, and provide experimental evidence for the role of endothelial function variability in the development of end-organ damage among healthy individuals.

The Concept of Endothelial Dysfunction

The concept of endothelial dysfunction has emerged from cardiovascular research over the past 25 years, recognizing the principal role of the endothelium in regulation of vascular function in healthy individuals and its impairment in diseased states.^{29,30} Endothelial dysfunction is now considered to play a principal role in the initiation and progression of atherosclerosis. Because endothelial dysfunction is also a common denominator for a wide variety of conditions such as hypertension, diabetes, or chronic renal failure, it may provide a link to increased cardiovascular risk in above-mentioned conditions.

Endothelial dysfunction may be defined as alterations in the normal properties of endothelium that are inappropriate for the preservation of organ function.³¹ Under physiologic circumstances, the endothelium maintains homeostasis at the vascular wall. Normal healthy endothelium reduces vascular tone, regulates vascular permeability, limits platelets adhesion and aggregation, prevents activation of the coagulation cascade, and restricts leukocyte adhesion. The specific term endothelial activation denotes the loss of endothelial anti-inflammatory properties characterized by elevated expression of adhesive molecules, such as E-selectin, intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1) and chemoattractant molecules, for example, monocyte chemoattractant protein-1, and

consequently pronounced interaction with blood leukocytes. Functional properties of endothelium and involved mediators are summarized in Table 1. One of the most important mediators released by endothelium is NO.³² NO acts a potent vasodilator, inhibits inflammation, growth of vascular smooth muscle, and aggregation of platelets. Altered production and/or bioavailability of NO are frequently reported in the conditions associated with endothelial dysfunction. However, the mechanisms responsible for the development of endothelial dysfunction are not yet completely understood. Probably the convergence of traditional and nontraditional risk factors, genetic predisposition and local, yet unknown mechanisms all contribute to endothelial perturbations.³² Summary of the mechanisms playing a role in endothelial cell dysfunction is given in Table 2.

Several methods are available for investigation of endothelial function in humans, which however assess only certain aspects of endothelial function. Measurements of endothelium-dependent vasodilation assess determines abilities of endothelium in coronary, forearm resistance, or brachial arteries. Transcapillary escape rate of intravenously injected radioactive albumin is employed as a surrogate for endothelial microvascular permeability. Finally, plasma levels of endothelium-derived regulatory mediators, such as Von Willebrand factor (vWF), soluble thrombomodulin, tissue-type plasminogen-activator (t-PA), plasminogen activator inhibitor-1 (PAI-1), soluble adhesive (selectins, ICAM-1, VCAM-1) and chemoattractant molecules (monocyte chemoattractant protein-1) could be used to estimate systemic endothelial activation. The current evidence for the association of microalbuminuria with several aspects of endothelial dysfunction in humans is summarized below.

TABLE 1. Properties of Endothelium and the Underlying Mediators

Regulated Process	Action	Mediators
Vascular tone	Vasodilators	NO Prostacycline Endothelium-derived hyperpolarizing factor (EDHF) C-natriuretic peptide (CNP) Bradykinine
	Vasoconstrictors	Endothelin-1 Thromboxane A ₂ Prostaglandin H ₂ Angiotensin II Reactive oxygen species
Permeability	Regulators	Endocytic receptors Cell surface glycocalix
Hemostasis	Anticoagulant or antithrombotic factors	NO Prostacycline Thrombomodulin Tissue factor inhibitor (TFI)
	Procoagulant or prothrombotic factors	Endothelin-1 Thromboxane A ₂ Reactive oxygen species Tissue factor (TF) vWF Fibrinogen
Inflammation	Profibrinolytic factors Antifibrinolytic factors Adhesion molecules	t-PA PAI-1 E-selectin ICAM-1 VCAM-1
	Chemoattractant molecules Antiproliferative factors	Monocyte chemoattractant protein -1 (MCP-1) NO Prostacyclin
Smooth muscle cell growth	Proliferative factors	Transforming growth factor β (TGF β) Platelet-derived growth factor (PDGF) Angiotensin II Endothelin-1 Reactive oxygen species Vascular endothelial growth factor (VEGF)

ENDOTHELIAL DYSFUNCTION PRECEDES THE DEVELOPMENT OF MICROALBUMINURIA

Increased Vascular Permeability

Increased permeability of albumin through the vascular wall is considered to be a marker of endothelial

dysfunction. The evidence that increased albumin leakage in the glomerulus is associated with enhanced capillary permeability for albumin in the systemic vasculature comes from several studies testing ¹²⁵I-albumin escape rate in diabetic microalbuminuric patients. Feldt-Rasmussen et al³³ showed that patients with type 1 diabetes and microalbuminuria exhibit higher transcapillary leakage of albumin than those without microalbuminuria. Similar findings were later reported in type 2 diabetic patients.³⁴ Furthermore, one study demonstrated that microalbuminuria was related to systemic vascular leakage even in healthy subjects.³⁵ Although microalbuminuria seems to be consistently associated with increased systemic leakage of albumin, some studies found increased albumin escape rate also in the hypertensive subjects with normoalbuminuria,^{36,37} suggesting that increased permeability of systemic microvessels is not always reflected in glomerular protein leakage. Although it is difficult to draw final conclusion from these findings, it might be suggested that in these states development of systemic vascular protein leakage actually precedes the appearance of microalbuminuria.

TABLE 2. Classical Risk Factors and Putative Mechanisms Leading to Endothelial Dysfunction

Classical risk factors
Aging
Hypertension
Smoking
Dyslipidemia
Obesity
Diabetes mellitus
Molecular mechanisms
Reduced bioavailability of NO
Oxidative stress
Hyperhomocysteinemia
Asymmetric Dimethylarginine
Angiotensin II
Advanced glycation end-products (AGE)

Impaired Systemic Endothelium-dependent Vasodilation

Another aspect of systemic endothelial dysfunction, the loss of vasomotor control in the peripheral vessels has been repeatedly found in microalbuminuric patients. Most of the data support the hypothesis on generalized endothelial dysfunction in albuminuria. In patients with diabetes type 1^{38,39} and type 2^{40,41} the endothelium-dependent dilation of the peripheral arteries is impaired in microalbuminuric subjects when compared with normoalbuminuric or healthy subjects. Furthermore, the presence of microalbuminuria is inversely related to flow-mediated dilation of the brachial artery in insulin-dependent diabetic patients,⁴² in elderly individuals with or without diabetes⁴³ and in asymptomatic type 2 diabetics.⁴⁴ Intriguing is the fact that these findings could be extended from high-risk populations to cohorts with clinically healthy subjects. Clausen et al⁴⁵ found that brachial artery flow-dependent dilation was significantly impaired in healthy individuals with microalbuminuria as compared with those with normoalbuminuria. However, it should be noted that some studies failed to find direct relationship between endothelial dysfunction and urinary albumin excretion in essential hypertensives⁴⁶ and healthy subjects.⁴⁷ In fact, blunted systemic endothelium-mediated dilatory response is often found also in normoalbuminuric diabetic subjects,^{48–50} strongly suggesting that impaired endothelial function precedes the development of microalbuminuria.

Elevated Plasma Levels of Pro-thrombotic and Pro-inflammatory Endothelial Markers

Increased plasma levels of inflammatory and prothrombotic markers have also been reported from the various cohorts of patients with microalbuminuria suggesting that elevated urinary excretion of albumin is associated with generalized endothelial activation and a low-grade inflammatory state. Higher circulating levels of vWF were found in patients with hypertension and microalbuminuria as compared with those without microalbuminuria.⁵¹ In addition to vWF, other markers of a procoagulant state, such as PAI-1 and coagulation factor VII are elevated in both insulin-dependent⁵² and non-insulin-dependent diabetic patients^{53,54} with microalbuminuria. Therefore, systemic hemostatic dysfunction is frequently present in microalbuminuric patients. Elevations of vWF levels are also paralleled by increased markers of oxidative stress in type 2 diabetic microalbuminuric individuals.^{55–57} Activation of endothelial cells is characterized by excessive levels of soluble adhesive molecules (ICAM-1, VCAM-1, and E-selectin) and is present in microalbuminuric type 1^{58,59} and type 2⁶⁰ diabetics. Similar findings have also been reported in low-risk populations. Agewall et al⁶¹ showed that plasma levels of PAI-1 were independently related to the level of urinary albumin in healthy subjects in a cross-sectional manner.

Intriguing data from several groups provide compelling evidence that endothelial dysfunction might

actually precede the occurrence of microalbuminuria. In a longitudinal study performed in patients with type 1 diabetes, Stehouwer et al⁶¹ demonstrated that increases in vWF levels precede the appearance of microalbuminuria by approximately 3 years.⁶¹ Similar findings were reported in prospective study by Verrotti et al⁶³ in children with type 1 diabetes. Furthermore, baseline levels of vWF were strongly related to the de novo development of microalbuminuria in the follow-up of non-insulin-dependent diabetic cohort.⁶⁴ Comparable data have also been found in a low-risk population. In a 4-year prospective study performed in healthy subjects, baseline elevated levels of vWF and tissue plasminogen activator (t-PA) predicted the development of increased urinary albumin excretion.⁶⁵ Simultaneously to impaired endothelium-dependent dilation, increased levels of soluble adhesive molecules are already present in normoalbuminuric diabetic subjects, a finding consistent with the hypothesis on the endothelial dysfunction appearing before microalbuminuria.⁶⁶

In conclusion, microalbuminuria is associated with increase in systemic albumin permeability, impaired endothelium-vasodilation of systemic vasculature, and elevated levels of proinflammatory and prothrombotic endothelium-derived mediators. This is not only true in diabetic and hypertensive patients, but also in healthy subjects. Prevailing evidence suggests that in some of these states endothelial dysfunction actually precedes the development of microalbuminuria.

Prognostic Value of Endothelial Function for Cardiovascular Risk

Present data consistently confirm that increased urinary excretion of albumin might be a useful integrated early marker of renal and cardiovascular risk. On the other hand, prognostic value of endothelial function is still a matter of debate. Since recognition of principal role of endothelial dysfunction in the development of atherosclerosis, several studies have been designed to investigate prognostic value of endothelial dysfunction for cardiovascular outcome. However, reports from microalbuminuric cohorts are scarce.

Most of the studies investigated endothelial dilatory reactivity as a marker of endothelial dysfunction. Coronary or peripheral endothelium-mediated response in patients with mild, moderate, or established coronary artery disease predicted the adverse event rates in several studies.^{67–70} Prognostic value of peripheral endothelial vasodilation has been also shown in patients with end-stage renal disease⁷¹ and chronic heart failure.⁷² The predictive value of endothelial function in patients with normal coronary angiograms,⁷³ cohort of essential hypertensives,⁷⁴ and hypertensive postmenopausal women⁷⁵ might suggest that this measurement could identify patients at risk at very early stage of the cardiovascular disease.

Alternatively, the prognostic value of endothelium-derived regulatory markers in plasma has been reported from several studies. Once more, the majority of studies

was performed in patients with known coronary disease. In these cases, vWF, t-PA,⁷⁶ PAI-1,⁷⁷ soluble ICAM-1,⁷⁸ and endothelin⁷⁹ were predictors of cardiovascular events. In one of the few studies with a low-risk population, Ridker et al⁸⁰ found elevated plasma levels of soluble ICAM-1 to predict the risk for future myocardial infarction in healthy men.

Studies investigating the predictive value of endothelial dysfunction in patients with microalbuminuria are sporadic and the relation between these 2 parameters in predicting the cardiovascular outcome is not straightforward. Jager et al⁸¹ have found plasma levels of VCAM-1 being predictive for cardiovascular outcome in type 2 diabetics independently from microalbuminuria, suggesting that increased plasma levels of VCAM-1 might reflect a different aspect of endothelial dysfunction than microalbuminuria. The same group showed that cardiovascular risk predicted by microalbuminuria is modified by the presence of elevated levels of vWF and retinopathy in type 2 diabetics, favoring the conclusion, that “benign” microalbuminuria (without concomitant presence of endothelial dysfunction) has more favorable prognosis than “malign” microalbuminuria (with endothelial dysfunction).^{82,83}

In conclusion, predictive value of the endothelial dysfunction for cardiovascular outcome has been shown mostly in high-risk patients. Although some data suggest that this relationship exists in low risk, healthy, or microalbuminuric populations, further studies will be needed to establish it conclusively.

Endothelial Function of Healthy Individual Predicts the Development of End-organ Damage

In accordance with the aforementioned data showing that microalbuminuria is preceded by generalized endothelial dysfunction, convenient estimates of endothelial dysfunction might be employed as a sensitive risk marker in general and healthy populations. Moreover, recent data suggest the absence of a cut-off value for the predictive value of urinary albumin excretion in cardiovascular risk and even values considered to be within normal ranges might be associated with adverse prognosis^{17,25,84} (Fig. 1). Therefore, it is tempting to speculate that a similar relationship exists with endothelial dysfunction for example, variation in normal endothelial function might already determine the risk for end-organ damage. To test this concept, we performed several animal experimental studies investigating the endothelial function in healthy individuals and its relation to the development of end-organ damage.

In several rat models of chronic renal failure, long-term renal damage is acutely induced by a well-defined noxe, such as subtotal nephrectomy or a standard injection of nephrotoxic drug. This relatively uniform injury results in highly variable renal damage among rats suggesting that some healthy individuals are more susceptible to end-organ damage than the others. We hypothesized that the variability in renal endothelial

function among healthy animals might be responsible for the differences in end-organ damage. To this end we removed one kidney from healthy animal and measured endothelium-dependent vasodilation of small intrarenal arteries in vitro. Note, that endothelial vasodilatory function varied considerably among those healthy animals (Fig. 2). In addition, we measured the contribution of 3 principal endothelial dilatory mechanisms, namely, NO, endothelium-dependent hyperpolarizing factor, and prostaglandins by using corresponding inhibitors of these pathways. Again, the contribution of those mechanisms to the renal vasodilation was highly variable among healthy individuals (Fig. 2). After these measurements in vessels of the removed kidney, the additional noxe was applied to contralateral kidney such as infarction of 2/3 of the organ (model of subtotal nephrectomy) or standard intravenous injection of adriamycin (model of adriamycin nephrosis). As a consequence, progressive renal damage developed characterized by proteinuria and focal glomerulosclerosis in these models. Interestingly, the endothelium-dependent vasodilation of small renal arteries measured in healthy kidney predicted the subsequent development of end-organ damage in both models⁸⁵ (Fig. 3). Notably, individuals with more pronounced endothelial NO-mediated dilation are protected against end-organ damage. These data suggest that variability in endothelial function among healthy individuals accounts for differences in sensitivity to end-organ damage. Whether these experimental results could be extrapolated to the human situation, remains unclear. However, if so, measuring renal endothelial function in healthy individuals may provide a novel marker for identifying those at risk for the development of end-organ complications. The variability in endothelial response among individuals may have a genetic basis. Interestingly, polymorphisms of

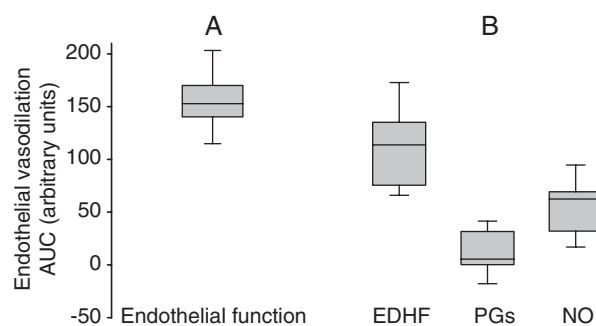
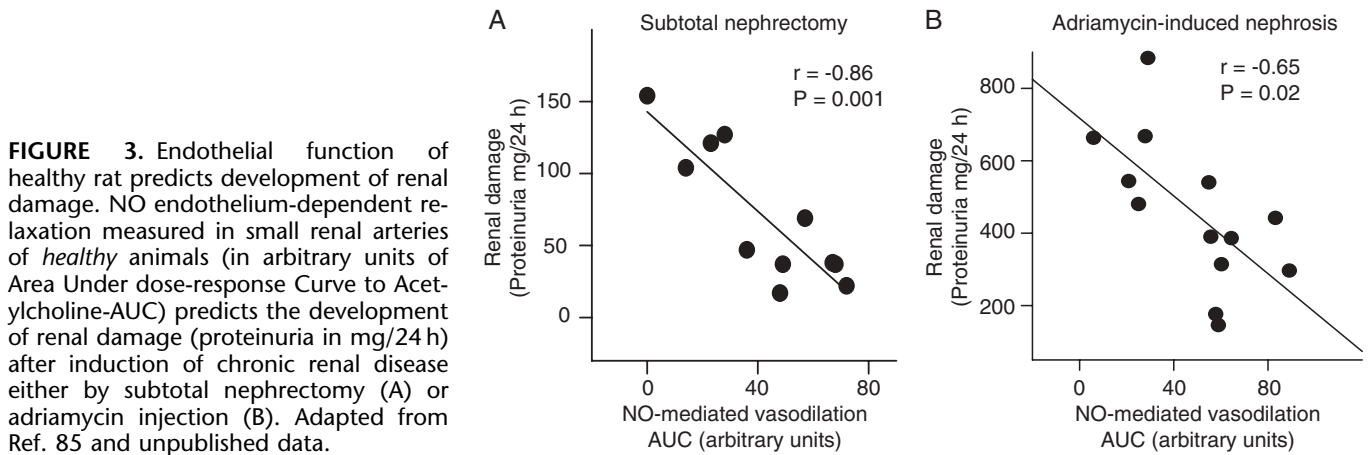


FIGURE 2. Variability in endothelial function (A) of small intrarenal arteries determined as endothelium-dependent vasodilation to acetylcholine and the contribution of endothelial vasodilatory mechanisms (B) in normal healthy Wistar rats. Horizontal line indicates median value, box encompasses 25th and 75th percentile and error bars correspond to 10th and 90th percentile. Note that endothelial function in healthy rat varies with a factor 2 (unpublished data). Data are expressed in arbitrary units of Area Under dose-response Curve to Acetylcholine-AUC; EDHF-endothelium-dependent hyperpolarizing factor, PGs-prostaglandins, and NO-nitric oxide.



several genes related to endothelial function, such as endothelial NO synthase⁸⁶⁻⁸⁸ or angiotensin-converting enzyme ACE^{89,90} have been implicated in end-organ damage. Still, endothelial function is a modifiable factor. Therefore, preventive modulation of renal endothelial function may represent a therapeutic target in healthy individuals with an increased risk to develop cardiovascular or renal damage.

MICROALBUMINURIA AND ENDOTHELIAL DYSFUNCTION AS THERAPEUTIC TARGET

Presence of microalbuminuria is consistently associated with worse cardiovascular outcome in several diseased conditions and in the general population. Therefore, it is of importance to explore, whether limiting of microalbuminuria provides benefit for decreased cardiovascular risk. Given the early occurrence of endothelial dysfunction in microalbuminuric patients, modulation of endothelial function might provide an additional strategy to limit adverse cardiovascular events. Furthermore, both the parameters emerge as therapeutic targets for primary prevention in the general population.

Lowering of Albumin Excretion is Associated With Reduction of Cardiovascular Risk

Several therapeutic approaches reverse the excessive urinary excretion of proteins. Strict glucose control may prevent the development of microalbuminuria in diabetic patients.⁹¹ Furthermore, several studies showed that angiotensin-converting enzyme inhibitors (ACEi),⁹²⁻⁹⁴ angiotensin II AT1 receptor blockers (ARB),^{95,96} lipid-lowering drugs, such as statins^{97,98} or fibrates⁹⁹ and recently also oral glycosaminoglycane sulodexide^{100,101} all reduce or even regress microalbuminuria in patients with type 1 or type 2 diabetes. Tight blood pressure control is required to halt the progression of microalbuminuria in hypertensive patients; however, drugs interfering with renin-angiotensin-aldosterone system (RAAS) might provide more benefit than diuretic, β -blocking agents, or calcium channel blockers in lowering albuminuria.¹⁰²

Importantly, available evidence suggest, that specific lowering of microalbuminuria translates in the

reduction of renal and cardiovascular adverse events in several populations. Parving et al⁹⁵ showed that lowering of albuminuria with ARB irbesartan is dose-dependently associated with reduced progression to diabetic nephropathy in hypertensive type 2 diabetics independent of blood pressure control. Comparably, several other studies demonstrated the efficacy of ACEi in preventing diabetic nephropathy in diabetic microalbuminuric patients.^{92,103} In the LIFE study among hypertensive patients with left ventricular hypertrophy, a reduction in albumin excretion was inversely related to the risk of cardiovascular mortality and morbidity.¹⁰⁴ It seems that drugs interfering with the RAAS are superior to other antihypertensives, also in reducing cardiovascular events in microalbuminuric subjects. This is, however, largely on the basis of the data from hypertensive diabetic populations, which are known to have high incidence of microalbuminuria.¹⁰⁵⁻¹⁰⁷

Recent compelling evidence for microalbuminuria as a justified target for primary prevention comes from the PREVENT-IT study.¹⁰⁸ Healthy individuals with microalbuminuria, but without hypertension or hypercholesterolemia, were treated either with placebo or the ACEi fosinopril. At 4-year's follow-up, the microalbuminuria was effectively reduced by ACEi treatment, which was associated with a 44% reduction in cardiovascular events.

In conclusion, lowering of urinary albumin excretion, preferably by RAAS inhibitory agents substantially reduces the number of cardiovascular events in both high risk and healthy population.

Modulation of Endothelial Dysfunction in Microalbuminuric Patients

A wide spectrum of treatments (Table 3) has been shown to improve endothelial dysfunction in several conditions. However, the hypothesis, that reversal of endothelial dysfunction is associated with risk reduction has not been directly tested. Nevertheless, some of endothelium-protective therapeutic strategies, such as ACEi,¹⁰⁹ ARB,¹¹⁰ and statins¹¹¹ have been consistently shown to reduce cardiovascular events in multiple

TABLE 3. Intervention Strategies Leading to Reversal of Endothelial Dysfunction in Humans

General interventions
Physical activity
Smoking cessation
Lipid-lowering therapy
Glycemic control in diabetes
Pharmaceuticals
Angiotensin converting enzyme inhibitors
Angiotensin receptor blockers
Statins
Peroxisome proliferator-activated receptor- γ activators
Estrogens
Dietary supplements
n-3 fatty acids
Folate
Tetrahydrobiopterin
L-Arginine
Vitamin C
Vitamin E
Dietary flavonoids

populations. It is, however, unclear to what extent the improvement in endothelial function governs cardiovascular risk reduction.

A limited number of studies is available on reversing endothelial dysfunction in patients with microalbuminuria. Strikingly, all therapeutic approaches associated with lowering microalbuminuria are also known to improve endothelial function. Therefore, it is tempting to speculate that improvement of endothelial function plays a role in the beneficial effects of these drugs on albumin urinary excretion and probably in cardiovascular risk reduction. However, the current data from diabetic patients do not allow such conclusion on this issue. Several trials investigated the effect of ACEi and ARB treatment on the peripheral endothelial function in diabetic microalbuminuric patients. Although in type 1 diabetics Arcaro et al¹¹² found ACEi to improve endothelium-dependent vasodilation of femoral artery without affecting microalbuminuria, Schalkwijk et al¹¹³ reported unchanged peripheral endothelium-dependent dilation after short-term therapy with ACEi quinapril. However, in the latter study ACEi reduced plasma levels of soluble E-selectin, suggesting that some aspects of endothelial dysfunction were selectively improved. Similarly, reversal of elevated VCAM-1 levels paralleled the decrease of microalbuminuria by fosinopril in hypertensive type 2 diabetics.¹¹⁴ In contrast, a low dose of ARB losartan, which did not affect blood pressure, did not have any impact on peripheral endothelial dilation, although it reduced microalbuminuria.¹¹⁵ Overall, studies with RAAS interfering agents suggest that these drugs may improve several aspects of endothelial function in microalbuminuric diabetic subjects, but it is not clear whether these effects play role in their antialbuminuric action. Factors such as duration of treatment, population, and agent characteristics might underlie these discrepancies.

In addition to ACEi and ARB, one study investigated the effect of atorvastatin on brachial artery

flow-mediated dilation in microalbuminuric type 1 diabetics. Six weeks treatment improved vasodilation, but had no effect on albumin excretion, probably because of the short duration of the treatment.¹¹⁶ Therefore, also statins may prove beneficial in reversing endothelial dysfunction in microalbuminuric diabetic patients.

In conclusion, the available data provide evidence that lowering microalbuminuria especially by RAAS modulating agents in diabetics, hypertensives, and even in healthy subjects might provide benefits in terms of reduced cardiovascular events. Majority of the agents efficiently lowering microalbuminuria might also reverse endothelial dysfunction. However, a role of the endothelial modulation in risk reduction remains unclear. Nevertheless, experimental evidence suggesting that endothelial dysfunction precedes microalbuminuria and that variability in endothelial dysfunction among healthy individuals determines the end-organ damage renders endothelial dysfunction the important modifiable factor for primary prevention.

Future Potential of Dietary Flavonoids in Primary Prevention of Microalbuminuria

Data from the PREVENT-IT study clearly show that the reduction of microalbuminuria among healthy subjects might prevent future cardiovascular events. Although modulation of microalbuminuria and endothelial dysfunction might be the most efficiently achieved by ACEi or ARB, additional strategies might prove useful in primary prevention. Furthermore, as reported from PREVENT-IT cohort, in otherwise-healthy microalbuminuric population only 63% of subjects were compliant to ACEi treatment. Therefore, for primary prevention, dietary supplements might provide more acceptable and inexpensive alternative to pharmaceutical compounds.

Recently, attention has been drawn to several nutritional factors in prevention of cardiovascular disease. Majority of the research concentrated on n-3 fatty acids, antioxidant vitamins, L-arginine, folic acid, and plant-derived polyphenols. The latter can be present in relatively high concentrations in certain plant-based foods and beverages, such as red wine, tea, grapefruit juice, or cocoa-based products.¹¹⁷ This makes them potentially interesting candidates for primary prevention. Several studies performed both in high and low-risk populations suggest beneficial effect of these supplements on cardiovascular outcome.^{118,119} These benefits seem to be mediated by improved endothelial function. Indeed, plant polyphenols, especially flavonoids have been shown to improve endothelial function in experimental and human studies.^{120,121} Even more important for primary prevention is the fact that endothelium-protective characteristics of flavonoids, such as increase in NO bioavailability due to antioxidant properties or stimulating effects on endothelial NO synthase, have been found in healthy individuals. This suggests the potential of flavonoids to modify healthy endothelial function and thereby modulate individual sensitivity to cardiovascular injury.

Although the flavonoids content is variable among the various dietary products and is also dependent on food processing practices, it seems that raw cocoa might contain the concentrations of specific flavonoids substantially exceeding most other known sources.¹²² Beneficial vascular effects of cocoa-based products, probably attributed to subclass of flavonoids known as flavanols, has been recently extensively reported.^{123,124} Impressive studies in healthy humans have shown that ingestion of flavanol-rich cocoa is associated with increased NO-dependent vasodilation,¹²⁵ reduced wave reflections,¹²⁶ decrease in blood pressure, and even an increase in insulin sensitivity.¹²⁷ Beneficial effects on endothelial function and insulin sensitivity were also confirmed in smokers¹²⁸ and hypertensives.¹²⁹ Although the effects of cocoa flavanols on renal vasculature and albumin excretion await further investigation, dietary strategies using flavanol-rich cocoa hold a promise as a primary preventive approach in subjects with microalbuminuria.

CONCLUSIONS

In conclusion, **microalbuminuria is an early and sensitive marker** of renal and cardiovascular risk in both high and low-risk patients. Most likely, it reflects a state of **generalized endothelial dysfunction**. Modulation of microalbuminuria and endothelial function might provide beneficial effects on future cardiovascular outcome even in the general or healthy population. Therefore, targeting microalbuminuria and endothelial dysfunction by several agents, such as ACE inhibitors or angiotensin receptor blockers might provide an effective strategy for primary prevention of renal and cardiovascular disease. Inexpensive and well tolerated dietary strategies using plant-based products such as flavanol-rich cocoa might prove useful for primary prevention of end-organ damage in the general population.

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