

PREVEND

ASSESSING THE IMPACT OF MICROALBUMINURIA



GRONINGEN



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THE SECOND SURVEY

GRONINGEN, AUGUST 2005

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SCIENTIFIC REPORT

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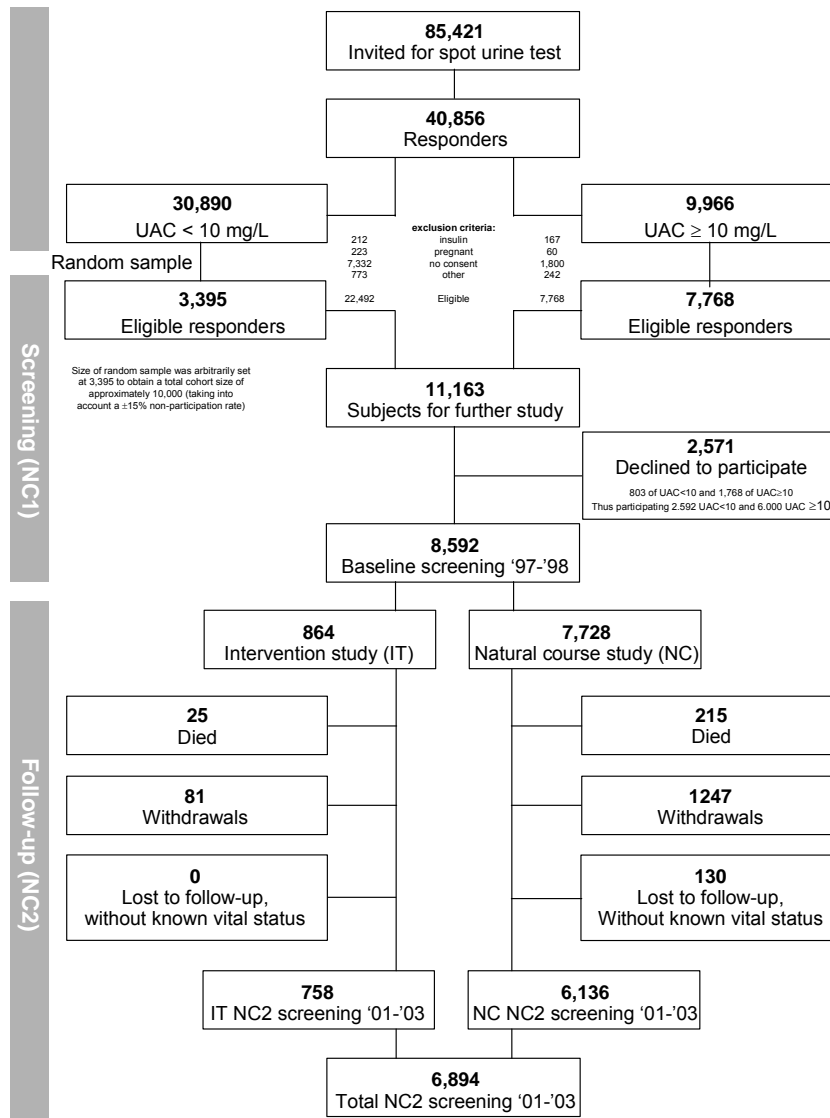
INTRODUCTION

The **Prevention of Renal and Vascular End-stage Disease (PREVEND)** study aims to study the impact of an elevated urinary albumin loss in non-diabetic subjects. We hypothesised that microalbuminuria is associated with a worse cardiac and renal survival, and that lowering of albuminuria improves renal and cardiac survival. PREVEND also aims to show whether it is worthwhile to implement screening for albuminuria in the general population.

Three years ago we published our report: PREVEND: the First Survey. As now part of the data of the second survey have been analysed and published, in the present report we will focus on the progress that has been made since 2002.

PREVALENCE OF MICROALBUMINURIA AND IMPAIRED GFR

The first aim of PREVEND was to study the prevalence of microalbuminuria in the general population. This was done in an a-select sample of 40,856 subjects of the Groningen population (see paragraph "Logistics" and figure 1).



It was shown that microalbuminuria (given here as a urinary albumin concentration (UAC) of 20-200 mg/L) was present in 7.2%, while 16.6% had high normal albuminuria (10-20 mg/L), and 0.7% macroalbuminuria (>200 mg/L) (9). Of even greater interest was the finding that 75% of the 2919 subjects that were found to have microalbuminuria, did not have any of the diseases known to underlie microalbuminuria, i.e. diabetes or hypertension (9) (figure 2).

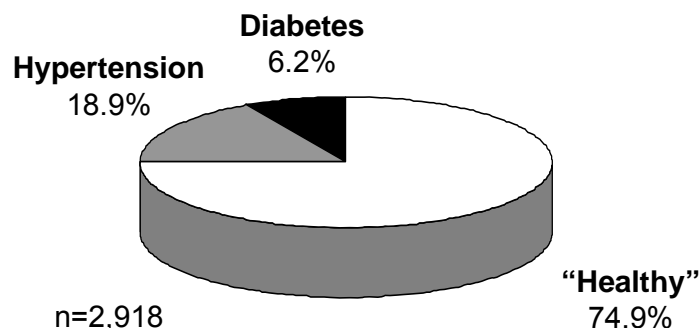


Figure 2. The percent of subjects diagnosed with microalbuminuria, according to known history of diabetes, hypertension, or no specific underlying cause. Most of the microalbuminurics were not known with a diagnosis of hypertension or diabetes.

In the PREVEND cohort consisting of 8,592 subjects we also measured creatinine excretion in two 24-hour urine collections to calculate creatinine clearance. We found 23% of the subjects to have a creatinine clearance (CCr) >90 ml/min, 71% a CCr of 60-90, 5.3% a CCr of 30-60 ml/min, 0.1% a CCr of 15-30 ml/min, and 0.1% a CCr of <15 ml/min (61). The finding that microalbuminuria can be considered as one of the earliest manifestations of chronic kidney disease (CKD) was well taken into account in the recent publications of the KDOQI guidelines describing the 5 stages of CKD. These stages are defined by the level of eGFR, being >90, 60-90, 30-60, 15-30 and <15 ml/min/1.73m² for the stages 1 to 5, respectively. In the stages 1 and 2 micro-albuminuria must be present, while in the other three stages the presence of microalbuminuria is not obligatory. This implies that subjects with the earliest phases of renal damage can only be detected by screening for microalbuminuria. Using the PREVEND database, we showed that about 10% of the general population can be regarded as having any of these stages of CKD (table 1) (61). These figures are close to those of the NHANES database.

| Stage | GFR | Albuminuria | Netherlands PREVEND % | USA NHANES % |
|-------|-------|-------------|--------------------------|-----------------|
| 1 | >90 | Yes | 1.3 | 3.3 |
| 2 | 60-90 | Yes | 3.8 | 3.0 |
| 3 | 30-60 | yes/no | 5.3 | 4.3 |
| 4 | 15-30 | yes/no | 0.1 | 0.2 |
| 5 | <15 | yes/no | 0.1 | 0.2 |

Table 1. The percent subjects in the PREVEND study and in the NHANES study according to the various stages of chronic kidney disease.

RISK FACTORS FOR MICROALBUMINURIA AND IMPAIRED GFR

Since so many subjects appeared to have microalbuminuria, but were not known with diabetes and hypertension, we tried to unravel which factors are responsible for the presence of microalbuminuria. We showed that other known CV risk factors, such as obesity (12,25) and smoking (5,11), but also specific drugs such as oral contraceptives and hormone replacement therapy (10) are associated with a greater risk for microalbuminuria, while not all antihypertensive drugs seemed to reduce urinary albumin excretion equally effectively (19).

Our studies on risk factors for impaired GFR were more difficult to interpret, as we found a bimodal relation between microalbuminuria and GFR. This phenomenon is discussed separately under the heading: glomerular hyperfiltration and microalbuminuria (see below).

MICROALBUMINURIA AND ELEVATED hsCRP

While in the past decade much attention of the nephrologist was directed to the prognostic value of microalbuminuria for renal and cardiovascular damage, cardiologists were fully focussed on the impact of an elevated high sensitive C-Reactive Protein (CRP) level on cardiovascular prognosis (Ridker PM NEJM 1997;336:973-979). Indeed CRP and microalbuminuria bear many similarities in that respect. We found a close parallel between UAE levels and CRP levels (73). Moreover, many of the risk factors for microalbuminuria are also related to a high level of CRP (67). After our publications that microalbuminuria, just as CRP (21,32), is associated with cardiac (2,8,13,15,16,30,44,50,55) and vascular damage (45) also cardiologists became aware of the usefulness of microalbuminuria as both a marker of CV risk and a target for treatment (43,61, 65).

Increased UAE is not only a manifestation of leakage of glomerular capillaries. It is considered a manifestation of generalised endothelial damage, manifesting itself in the kidneys (which however, makes detection easy) as well as in other vessels (Deckert T, Diabetologia 1989;32:219-226). The association between atherosclerosis and an elevated CRP level, a parameter of inflammation, is considered to be due to the fact that the generalised atherosclerotic process in fact is a process of low grade vascular inflammation (Ross R. NEJM 1986;314:488-500). In that respect it is likely that microalbuminuria and a high CRP level add to each other to predict renal and vascular risk. We showed that CRP was negatively associated with GFR, suggesting that an impaired kidney function is associated with an inflammatory process (26). Of interest also was the data that in subjects with an elevated CRP level the association between blood pressure and albuminuria is more steep. Inflammation thus seems to increase the glomerular leakage of albumin in response to blood pressure (32). We moreover showed that CRP and albuminuria are independent and additive risk predictors for all-cause and cardiovascular mortality. Both markers provide additional information about CV risk than conventional risk factors alone (48, 67).

MEASUREMENT TECHNIQUES TO DETECT EARLY RENAL DAMAGE

Creatinine clearance and estimated Glomerular Filtration Rate

As it has been widely accepted that an impaired kidney function is a risk factor for progressive CVD, many epidemiological studies presently also measure renal function. The PREVEND study is one of the first to have data on kidney function in so much detail (not only serum creatinine and GFR estimates based upon a serum creatinine measurement, but also actually measured creatinine clearance obtained from two 24hr urine collections), of so many subjects, and measured serially. This enabled us to publish reports on the accuracy of GFR

estimates in screening studies (28,35,58,64), and on the use of cystatin C, a newly proposed marker of renal function (34,53).

We showed that the use of GFR estimates in epidemiological studies results in incorrect conclusions on the impact of risk factors on renal function. We showed that the relation between age and renal function is completely different when using the Cockcroft-Gault formula, the MDRD formula or actually measured creatinine clearance (figure 3) (35).

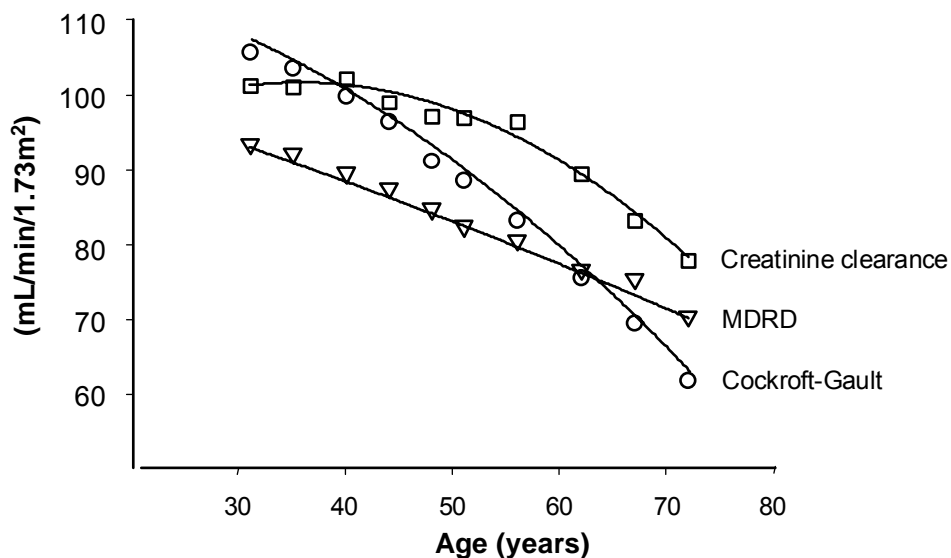


Figure 3. *The relation between age and renal function, when renal function is expressed as 24-hour creatinine clearance, or as Cockcroft Gault- or MDRD- estimated GFR. The conclusion on the impact of age on renal function decline over age is completely different regarding the method used.*

In a cooperation with the Harvard epidemiology group we showed that cystatin C cannot be used as an accurate marker of GFR, as serum levels are not, as always claimed, independent of other anthropometric measures, such as age, sex, weight, and height (34). This led us to conclude that, to be able to use cystatin C serum levels as a marker for GFR, formulas should be used that take into account factors that relate to the production of these substances, just like it is with serum creatinine (53). This conclusion was also prominent after our analysis of cystatin C as an indicator of CV-events. We showed that the association between cystatin C levels and CV-events is independent of renal function. This indicates that, though cystatin C is related to CV-events, this is not due to the fact that cystatin C reflects renal function adequately (86). The use of GFR estimates in epidemiological studies has also been discussed in recent reviews (58,63,64).

Urinary albumin excretion

Two aspects have been studied, e.g. the quality of laboratory measurement of albumin in the urine, and the best method to express the amount of albumin in the urine.

Many epidemiological studies at present also want to include albuminuria in their dataset. To that purpose also frozen samples are being used. We tested whether the freezing procedure itself and the duration of storage of frozen urine influences the result. It was shown that long-term storage of urine at -20°C results in a steady decline in assayable albumin, reaching a plateau at a decline of about 30% beyond one year of storage. Another point that we

observed was that intra-individual variation in urinary albumin concentrations increased (56). A comparison between UAE obtained from fresh samples with UAE obtained from samples that had been stored frozen for more than 2 years at -20°C as predictors of mortality revealed that frozen samples still predict mortality very well, but slightly less well than fresh samples (83).

Albuminuria measurements are based on an immunoassay. This means that only immuno-reactive albumin in the urine is being measured. Recently a HPLC technique to separate albumin became available. We tested the ability of this method to detect microalbuminuria in a sample of the PREVEND population, enriched for the presence of elevated albuminuria levels. We showed that the HPLC method detected twice as many subjects with an albumin excretion in the microalbuminuric range than the classical nephelometry, while the number detected with macroalbuminuria was comparable in both groups. Interestingly, the (larger group) of patients detected with the HPLC had a similarly impaired ankle brachial index as the smaller group detected with the nephelometry, suggesting that we detected subjects with an equally impaired vascular function that would otherwise not have been detected (39).

We also tested what the best method of urine collection would be and what the best approach is to screen for abnormal urinary albumin excretion (41). Of course the gold standard is the amount of albumin in 24-hour urine collections, preferably in two or three collections. This is the approach followed in PREVEND. This however requires good instruction and motivation of the subject to guarantee an optimal urine collection. As this, especially in mass screenings, is difficult to achieve, we tested how a screening using an approach of just a simple spot morning urine sample can reliably detect subjects who indeed prove to be microalbuminuric in subsequent 24hr urine samples. We found that the diagnostic performance of measuring UAC in a morning urine sample to predict a UAE>30mg in subsequent 24-hour urine collections is satisfactory and comparable to that of measuring albumin/creatinine ratio (ACR) (figure 4).

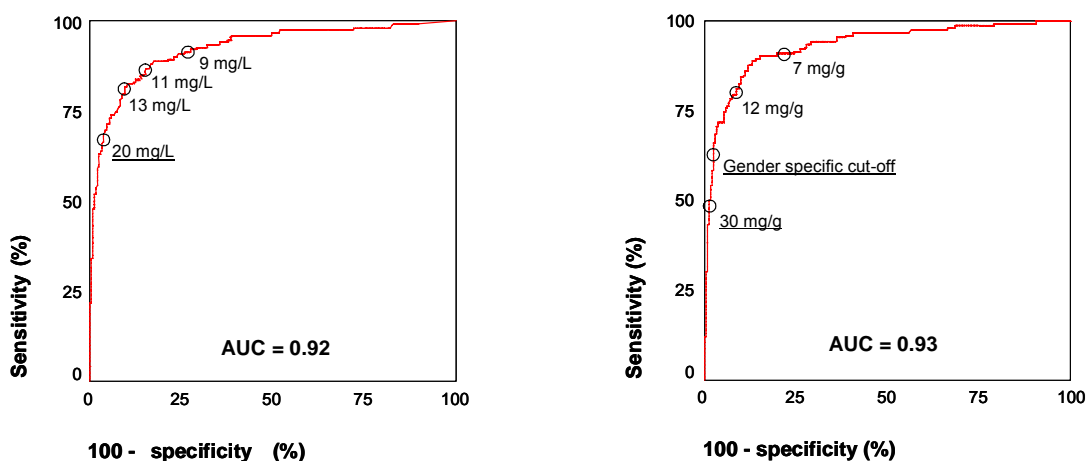


Figure 4. ROC curves showing the diagnostic performance of a spot morning urinary albumin concentration (UAC) (left) or albumin creatinine ratio (ACR) (right). ACR is not superior to UAC.

The best way to have the false negative rate as low as possible is to use a cut-off value of 10 or 11 mg/L (47). These findings contrast with the frequently expressed advice of using an ACR to detect subjects with microalbuminuria. We do not support that advice for mass screening (57,58), as this introduces bias due to the measurement of another substance, e.g. creatinine. Moreover, it has been well proven that creatinine excretion in the urine is about 40% higher in men than in women, and also about 20% higher in young subjects than in

elderly ones (20). These aspects of the measurement of albuminuria have also been described in various reviews (57,58,63,65).

A final aspect addressed is the definition of microalbuminuria as a fixed cut-off value. Despite we have shown that no specific cut-off level can be identified for the risk attributed to an increased urinary albumin excretion (17), we think it is of great use to specify a cut-off (see also in the paragraph on mass screening, later in the manuscript). We concluded that one should consider a urinary albumin excretion as abnormally high at a level above which trials have shown that treatment provides a clinically significant benefit versus no treatment. Of course, also cost-effectiveness should be taken into account (46). Until these issues are resolved, we should continue to use the current definition for microalbuminuria. This allows correct interpretation of data of the past. Of note, in our intervention study in apparently low risk subjects we showed such a benefit to be clear above an UAE of 50mg/day (40). These data have yet to be corroborated though.

GLOMERULAR HYPERFILTRATION AND MICROALBUMINURIA

The first studies on the relation between albuminuria and GFR showed a striking biphasic response. While high normal albuminuria and the early phases of microalbuminuria were associated with glomerular hyperfiltration, more pronounced elevations of albuminuria were associated with an impaired GFR (6). This biphasic pattern was even more strikingly observed when these associations were adjusted for confounders (52). This pattern is similar to that described in type 1 and type 2 diabetes, which suggests a parallel between the mechanisms relating microalbuminuria to renal prognosis (42). It is therefore likely that glomerular hyperfiltration precedes a period of microalbuminuria. At the time that microalbuminuria is manifest, GFR starts to decrease, not to return to normal levels, but to progressively fall to the levels of ESRD.

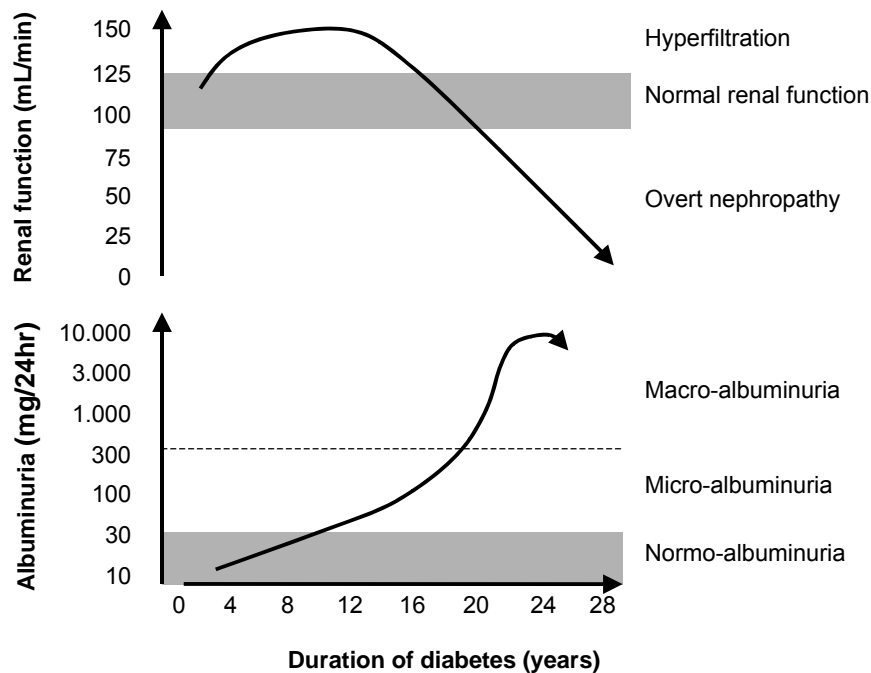


Figure 5. *The course of renal function over the years of diabetes duration. Initially normoalbuminuria is found. Glomerular hyperfiltration is associated with the phase of microalbuminuria, followed by a progressive fall in GFR with macroalbuminuria (Thesis JC Verhave).*

Interestingly, we also observed a biphasic relation between systolic blood pressure and renal function, and between plasma glucose and renal function, with glomerular hyperfiltration being manifest at completely normal blood pressure and glucose levels (27). This is in line with our finding that slight elevations in blood pressure, plasma glucose, and body mass index, which are well within the range currently accepted as normal (SBP<140 mmHg, plasma glucose <6.1 mmol/L and BMI <25kg/m²), are also associated with an increased UAE (27). This suggests that some subjects are more sensitive for the detrimental effects of these CV risk factors, in the sense that they already develop vascular damage, manifest as glomerular hyperfiltration and elevated albuminuria, with levels of these risk factors currently accepted normal.

We studied whether this enhanced sensitivity for the effects of risk factors on albuminuria and renal damage is influenced by genetic and environmental factors. We showed that higher salt intake is associated with higher albuminuria, and that there is an interaction between body mass index and obesity in this effect of salt intake on albuminuria: with a higher BMI the relation between sodium intake and albuminuria is more steep (37).

A next question to address would be to explain the association between glomerular hyperfiltration and albuminuria. We hypothesised that the association between the duration of diabetes and GFR/albuminuria (figure 5) may also be found in the normal process of ageing (42), independent thus of the presence of manifest hypertension and diabetes. We questioned whether that may be the consequence of insulin resistance. We therefore studied the impact of insulin resistance (measured as insulin levels) on the age-associated changes in albuminuria and renal function. We found that in subjects with high insulin levels (thus insulin resistant subjects) glomerular hyperfiltration is present, most prominent at young ages, and that with advancing age albuminuria levels increase and creatinine clearance decreases more in these insulin resistant subjects (87).

We also tested whether genetic factors may influence albuminuria and renal function. We found that genetic variation in the endothelin 1 gene has a significant effect on GFR but not on UAE. (24). We also found that carriers of the cytochrome-P450 3A5*1 allele have a significantly lower blood pressure, an effect that was modulated by sodium intake (59).

THE FOLLOW-UP FINDINGS

Urinary albumin excretion in relation to progressive renal and CV disease

Our initial focus was directed to study the changes in renal and cardiovascular function in the subjects with an elevated albuminuria as compared to those without. We showed that microalbuminuria is associated with a worse renal survival: the number of subjects developing a de novo eGFR<60 ml/min increased with a higher baseline albuminuria value (38) (figure 6).

Momentarily, preliminary analyses are performed using data on eGFR from the first and second, but also the third screening that presently is running. Data are available yet of 3,429 subjects. With linear regression analysis, using these three eGFR values, a slope of renal function decline over time can be calculated for each individual. This analysis again shows that baseline albuminuria is a good predictor of the rate of renal function decline, and better than conventional atherosclerosis risk factors, such as blood pressure and cholesterol. We next documented that subjects with microalbuminuria have a worse survival than those without (17). It was shown that each twofold increase in UAC results in a 1.29 higher risk for CV death. It was also shown that the risk encountered with microalbuminuria is about twofold higher than the risk encountered with having hypertension, diabetes, hyperlipidemia, obesity or smoking (14).

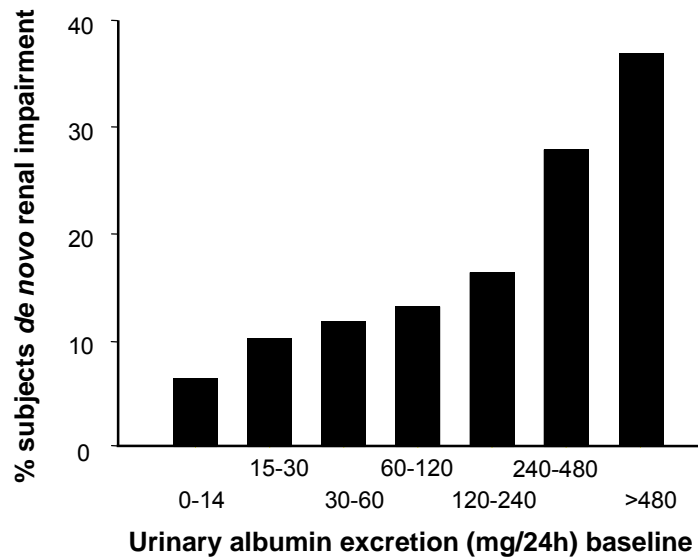


Figure 6. *The percent subjects with de novo development of an impaired GFR in relation to baseline albuminuria.*

Currently, it is being investigated to what extent new cardiovascular risk markers such as urinary albumin excretion, CRP, the prohormone of brain natriuretic peptide (proBNP), serum creatinine, and cystatin C compare to each other and to traditional cardiovascular risk markers with respect to prediction of cardiovascular morbidity and mortality. Preliminary data revealed that the new cardiovascular risk markers are independent of each other and have better predictive properties than the traditional ones, with exception of age and smoking status. We foresee in the near future to propose a “Groningen Risk Score” in which urinary albumin excretion and renal function parameters take prominent roles, and replace some of the more traditional risk markers.

Urinary albumin excretion in relation to new diabetes and hypertension

As such these data on renal and CV outcome confirm our baseline hypothesis, that it may be of great benefit to detect subjects with microalbuminuria. We however, also studied whether screening for microalbuminuria may also help to detect subjects at risk to develop diabetes and/or hypertension. This hypothesis may at first not seem logical, because microalbuminuria is usually considered to be a complication of diabetes mellitus rather than a cause. However, the hypothesis is based upon our finding that microalbuminuria is already elevated with levels of plasma glucose and blood pressure that are in the completely normal range. Moreover, microalbuminuria has been argued to be part of the metabolic syndrome and, as we described above, is also strongly related to insulin resistance (85). That led us to hypothesise that one should also consider whether albuminuria precedes the development of diabetes or hypertension. Indeed, we found that subjects with microalbuminuria have an increased risk to develop diabetes (62) and hypertension (68). These findings are in line with recent reports that high sensitive CRP, a marker of low-grade vascular inflammation, may also precede the development of diabetes and hypertension.

We can thus conclude that our data show that indeed in non-diabetic subjects microalbuminuria has comparable predictive value for indicating increased renal and cardiovascular risk as in diabetics. It moreover has been shown that the concept of

microalbuminuria as a consequence of diabetes and hypertension should be reconsidered: microalbuminuria may indicate a generalised condition, that makes a subject sensitive to develop the full blown pattern of insulin resistance, ultimately leading to manifest diabetes and hypertension.

LOWERING OF ALBUMINURIA

In the PREVEND Intervention Trial (PREVEND-IT) we studied whether lowering of albuminuria would result in a better prognosis (3). Overall 864 subjects with high normal UAE or microalbuminuria (UAE of 15-300 mg/day), only modestly elevated blood pressure (<160/100 mmHg) and plasma cholesterol (<8.0 mmol/l, and in case of a previous myocardial infarction <5.0 mmol/L) were enrolled. They were in a two by two factorial design treated with either pravastatin or placebo and fosinopril or placebo.

We found that the ACE inhibitor lowered UAE during the entire follow up period of 4 years, while the statin did not influence UAE (40). The lowering of UAE by the ACE inhibitor was associated with an, albeit not statistically significantly different ($p=0.098$), reduction in the number of cardiovascular events, while the statin did not influence the number of CV events. The favourable effect of the ACE inhibitor was clearly dependent on baseline UAE. In subjects with an UAE of 15-50 mg/d the ACEi reduced the number of events from 5.1 to 3.6% (RRR of 29%), while in those with an UAE of 50-300 mg/d the ACE inhibitor reduced the number of events from 13.0 to 5.2% (RRR = 60%) (figure 7).

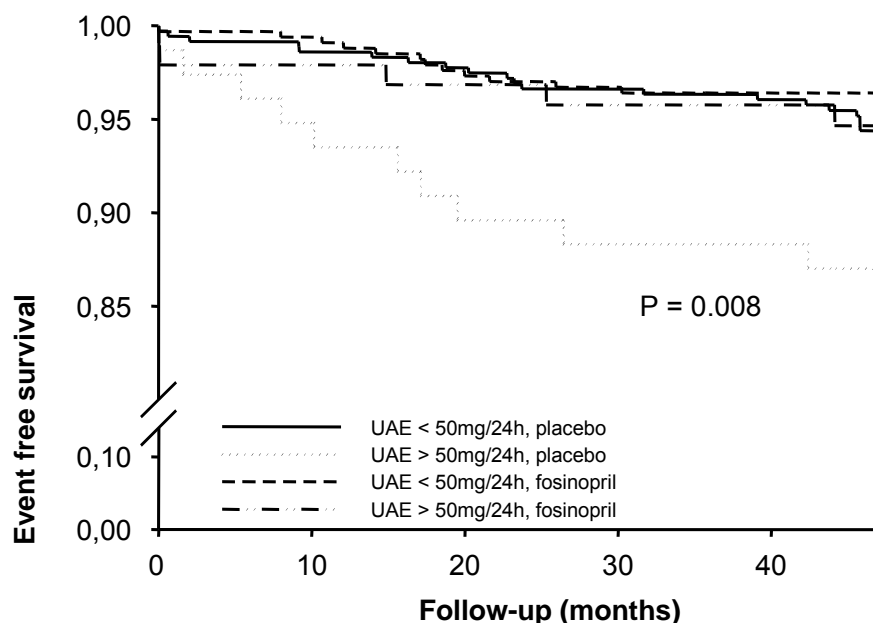


Figure 7. The effect of fosiopril versus placebo on cardiovascular events, divided by a UAE of more or less than 50 mg/day (40)

The finding that the statin did not lower albuminuria was in contrast to our expectations, as there were -at the time of the design of the study- some reports that had described a lowering of albuminuria on statins. Later however, some authors reported on an increase of albuminuria on statins. The latter was argued to be due to the consequence of the fact that statins may influence tubular albumin uptake, and thus may lead to a rise in the urinary loss of albumin. Such a blocking of tubular albumin uptake may be beneficial for long-term renal

function outcome as it has been argued that the presence of albumin in the renal interstitial tissue causes progressive interstitial fibrosis. We studied this both in the setting of a randomised controlled clinical trial (the PREVEND IT study) and in a daily clinical practice situation. We were able to do so because the PREVEND database is coupled to the InterAction DataBase (IADB) that registers drug use of all Groningen inhabitants. The use of that database offers a reliable information on drug use in an epidemiological survey (18). In the clinical trial, we found neither an effect of the statins on albuminuria nor on renal function decline. In the daily clinical practice setting of the PREVEND cohort, statin use in general was found associated with a (numerically small, but statistically significant) rise in albuminuria without a significant fall in GFR (71). This led us to conclude that statins may result in a rise in UAE, which is not associated with GFR preservation (71).

We also studied the factors that are related to a change in albuminuria over time. Interestingly, both baseline blood pressure and baseline plasma glucose, as well as changes in blood pressure were the most important factors related to a change in albuminuria. This indicates that a fall in pressure results in a lowering of albuminuria, independent of the way in which blood pressure is lowered, while a rise in pressure is associated with a rise in albuminuria. This tight relation between changes in pressure and changes in albuminuria is present also in a range of normal blood pressures (69).

IMPLEMENTATION OF ALBUMINURIA SCREENING PROGRAMS

The criteria needed to conclude that a screening program could be implemented in daily clinical practice are, according to Wilson-Jungner, the following:

1. The disease for which the screening test could be used, is an important health care problem
2. The natural course of the disease should be well described
3. The disease should be detected in an early phase
4. Treatment in an early phase should offer benefit
5. The test should be acceptable and there should be a well defined cut-off
6. The interval at which should be tested, should be known
7. The extra workload needed in case of a positive test should be acceptable
8. The risk of screening, both somatic and psychiatric, should outweigh the benefits
9. Screening and subsequent treatment in case of positive tests should be cost effective.

In fact, when considering a population screening program for albuminuria in order to lower renal and CV risk, most of these prerogatives are fulfilled. The topic sub 9 has also been taken into account in our program. We found that the screening of the Groningen population as carried out by us, and subsequent treatment of those found positive, as done in the PREVEND-IT study appeared to be cost effective (70). The costs needed to gain one life year were 16,559 euro. The threshold in the Netherlands to conclude an intervention is cost-effective is 20.000 euro. When we limited treatment to those with an albuminuria 50-300 mg/d, the costs needed to gain one life year were even only 7,030 euro.

These data are different from a recent report in the literature (Boulware et al JAMA 2003;290:3101-04). Those authors argued screening for proteinuria not to be cost effective. In that report, one however, performed a screening for dipstick positive proteinuria (which only detects macroalbuminuria), the screening was done via the routine practice of the general practitioner, and the effect of treatment was tested only on the prevention of ESRD. We tested on albuminuria by which we found more positive subjects, we did it via postal questionnaire which is much cheaper (see above sub logistics), and we tested treatment to prevent a CV endpoint which of course manifests more early than a renal endpoint (36). Based upon these data we can favour implementation of a screening program (58,63).

We found that subjects that have macroalbuminuria at the start of the study have a worse renal survival during 4 years of follow up, while subjects having an impaired GFR to start with have a renal prognosis not different from that in the background population. We therefore argue that it is better to detect subjects at risk by screening for albuminuria than for impaired GFR (84). To that purpose we more closely evaluated the approach used by us, in particular the option to carry out a pre-screening on one single morning urine sample to detect subjects who are positive using the gold standard, which consists of two 24hr urine collections. We showed that approach to have an acceptable sensitivity and specificity. We also showed that the risk of having too many subjects false negative could be minimised when using a cut off value of an UAC of 10 mg/L. We similarly showed that the use of an ACR does not have benefit over an UAC, thus limiting the costs, because the assessment of creatinine is not necessary. (47). We also evaluated whether this approach could help to detect subjects with undiagnosed diabetes, hypertension and hyperlipidemia. This seems indicated, as we in the PREVEND study also showed that one third of subjects who appeared to be hypertensive were not diagnosed before as such, and that of those known with hypertension one third was not adequately treated (1). We also showed that the likelihood to receive antihypertensive treatment increased when higher pressures were found, but was not higher in subjects with concomitant cardiovascular risk factors, suggesting that the approach of general risk factor profiling is not well implemented yet (33). The yield of detecting subjects with these -yet undiagnosed- risk factors was higher in case they were positive for albuminuria (81). Of course, many subjects with hypertension or hyperlipidemia, but not having an elevated albuminuria, would then have been missed. To see whether that would have much impact on the risk for CV events, we in addition studied whether subjects with undiagnosed hypertension, but without concomitant albuminuria, would have a worse survival. Undiagnosed hypertensives without albuminuria have a 50% increased risk on CV events compared to healthy subjects without hypertension or microalbuminuria, adjusted for age and sex. The risk of subjects with microalbuminuria was more increased, by 80% in case there was no hypertension, by 140% in case there was newly discovered hypertension, and even by 240% in case of previously known hypertension (82). These data combined suggest that population screening for microalbuminuria identifies a considerable number of previously unknown hypertensives at high risk for cardiovascular disease. A number of yet unknown hypertensives are “missed” by such screening, but fortunately they are at relatively low risk to develop a CV event during follow-up. Finally, it should be mentioned that the knowledge of being positive for microalbuminuria did not result in worse well being of the participants (7). Almost one third of the participants stated to live according to healthier principles afterwards.

CONCLUSIONS AND PROSPECTS FOR THE FUTURE

From the abovementioned we conclude, that

1. long term follow up of the PREVEND cohorts is feasible, considering a yearly drop out rate of less than 3%
2. the process of data collection and data storage is effective to draw firm and well validated scientific conclusions
3. these conclusions may have impact for daily clinical practice
4. the data not only offer interesting knowledge from an epidemiological perspective, but they also offer the opportunity to raise novel and exciting new pathophysiological concepts.

The data show that in non diabetic subjects:

1. an elevated UAE is found in considerable prevalence
2. an elevated UAE is to be considered as the earliest stage of chronic kidney disease
3. an elevated UAE indicates an increased cardiovascular risk

4. an elevated UAE may not only be the consequence of CV risk factors, such as diabetes, hypertension, obesity, and smoking, it may also precede manifest diabetes and hypertension.
5. glomerular hyperfiltration and elevated UAE seem part of the insulin resistance syndrome
6. screening for an elevated UAE may not only be effective to detect subjects at increased cardiovascular and renal risk, it is also a cost-effective approach
7. lowering of UAE in these non-diabetic subjects seems to prevent cardiac events

The data described in this report reflect the main outcome of the first 4 years of follow up. Presently the third screening is running (year 5-7). We are now preparing for the fourth screening, thus bringing the overall follow up to 10 years. It presently is widely appreciated that the PREVEND cohort is unique, in that in these subjects renal function is not only very accurately described, it moreover offers follow up data. There is no other cohort as this in the world, in which renal function is monitored longitudinally.

This follow up makes it possible to define the sequence of events leading to progressive renal function loss. A longer follow up with more data points will enable us to more precisely define the phenotype of subjects with progressive renal function loss in the absence of previous renal disease. That long-term follow up of our cohort, with more data points, is necessary to draw a longitudinal renal function curve. That data will greatly improve the power to conclude which factors contribute to the progressive loss of renal function. It is to be expected that it is the interaction between various classical risk factors with genetic and environmental factors that determine the progressive loss of renal function in some subjects. A longer follow up makes it also possible to better define the impact of modest renal damage for future progressive cardiovascular disease. It presently is not well understood why subjects with a stage 1 to 3 renal damage have an increased risk for CVD. As we have a large population of such subjects, and have them well defined regarding the renal and cardiovascular phenotype, our follow up will help to give such answers in the future. That long-term follow up will also lead to new, and sometimes unexpected, views on the pathophysiology of diseases, is clear from our studies that showed that microalbuminuria not only follows hypertension and diabetes, but may also precede it. Having various data points over time will enable to conclude to such phenomena. As such we also will evaluate to what extent microalbuminuria (as a reflection of vascular endothelial damage) and increased CRP (as a reflection of vascular inflammation) may add to each other, and what comes first?

PREVEND: AN EXPANDING NETWORK

While PREVEND in 1997 started as a cooperation between nephrologists, cardiologists, clinical pharmacologists and epidemiologists, the interests of other research groups in the study gradually increased. This was on the one hand due to the need to involve scientists with other expertise, and on the other hand interest of other research groups to answer specific questions using the PREVEND database.

Of specific interest also is the funding from the BreedteStrategie from the Groningen University, by which the genetic work in PREVEND was facilitated. It gave the possibility to combine genetic work on genes involved in progressive renal function loss in mice with studies in large databases with a well defined renal phenotype in humans.

In this way many researchers presently profit from the PREVEND know how. In the last paragraphs of this report the research groups that became involved in the PREVEND project are listed, both from our own university as those from abroad. In that respect many can also profit from our recent establishment of the Groningen random sample of 3,432 subjects. It can be used as a database to answer various questions, not disturbed by the original PREVEND design in which we enriched the population for the presence of albuminuria.

SCIENTIFIC OUTPUT AND ITS IMPACT

PUBLICATIONS

1999

1. Pinto-Sietsma SJ, Hillege HL, Janssen WMT. Inadequate management of blood pressure in a hypertensive population. *N Engl J Med* 1999; 340:1593-1595. [letter]

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70. Athobari J, Asselbergs FW, Boersma C, de Vries R, Hillege HL, van Gilst WH, Gansevoort RT, de Jong PE, de Jong-van den Berg LTW. Cost-effectiveness of screening for albuminuria and subsequent treatment with

- an ACE inhibitor to prevent cardiovascular events: a pharmaco-economic analysis linked to the PREVEND and the PREVEND IT studies. Submitted.
71. Atthobari J, Brantsma AH, Gansevoort RT, Visser ST, Asselbergs FW, van Gilst WH, de Jong PE, de Jong-van den Berg LTW. The effect of statins on urinary albumin excretion and glomerular filtration rate: results from both a clinical trial and an observational cohort study. Submitted
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 76. Rosmalen JGM, de Jonge P, Gans ROB, Neeleman J. Neuroticism and somatic ill health: more than symptom perception alone? Submitted
 77. Smilde TDJ, Zuurman MW, Hillege HL, van Veldhuisen DJ, van Gilst WH, van der Steege G, Voors AA, Kors JA, de Jong PE, Navis GJ. Renal function dependent association of AT1-receptor polymorphism (A1166C) and electrocardiographic left ventricular hypertrophy. Submitted.
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 79. De Jong PE, Hostetter T. Screening, monitoring and treatment of albuminuria; public health perspectives. J. Am Soc Nephrol, in preparation
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 82. Ozyilmaz A, Brantsma AH, Bakker SJL, de Zeeuw D, de Jong PE, Gansevoort RT. Should treatment of hypertension be guided by the presence of microalbuminuria? Abstract ASN 2005, manuscript in preparation
 83. Brinkman JW, de Zeeuw D, Gansevoort RT, Duker JJ, Kema IP, de Jong PE, Bakker SJL. Frozen or fresh urine samples for measurement of albuminuria as predictor of renal impairment and mortality? Abstract ASN 2005, manuscript in preparation
 84. Halbesma N, Brantsma AH, Wetzels J, Bakker SJL, de Zeeuw D, de Jong PE, Gansevoort RT. Macroalbuminuria is a better risk marker than low GFR to identify subjects at risk for accelerated GFR loss in a population screening. Abstract ASN 2005, manuscript in preparation
 85. Zuurman MW, Korstanje R, van der Steege G, de Jong PE, Navis GJ. CCL2-2518 promoter polymorphism and a high vascular risk score are associated with impaired renal function in the general population. Abstract ASN 2005, manuscript in preparation
 86. Brinkman JW, Bakker SJL, Gansevoort RT, de Zeeuw D, de Jong PE, Hillege HL. Cystatin C as a predictor of increased cardiovascular risk is independent of renal function: a large cohort study. Abstract ASN 2005, manuscript in preparation
 87. Oterdoom LH, de Vries AJ, Gansevoort RT, de Jong PE, Gans ROB, Bakker SJL. Fasting insulin concentrations modify the relation between age and renal function. Abstract ASN 2005, manuscript in preparation

The following theses have been prepared based upon the PREVEND database

1. 1997-2000 SaraJoan Pinto-Sietsma NSN 14-11-2001
Risk Factors for Renal Abnormalities in a Non-diabetic Population
2. 1997-2000 Gilles FH Diercks NHS/industry 30-01-2002
Microalbuminuria. A cardiovascular risk indicator
3. 1998-2000 Hans L Hillege 1^e 16-09-2002
Renal Risk Markers and Cardiovascular Disease
4. 1998-2001 Taco BM Monster 1^e 31-01-2003
Assessing Drug Influences on Urinary Albumin Excretion
5. 2000-2003 Jacobien C Verhave NSN 10-11-2004
The Impact of Albuminuria and Cardiovascular Risk Factors on Renal Function
6. 2000-2003 Eric M Stuveling NSN 15-12-2004
CRP and Albuminuria. Independent Risk Markers of Renal and CV Disease
7. 2000-2003 Folkert W Asselbergs NHS/industry 12-01-2005
Vascular Function and Cardiovascular Disease

Under preparation are theses by

8. 2002-2005 Susanne E Borggreve NHS
Genetic variation in CETP, reverse cholesterol transport and cardiovascular risk
9. 2003-2006 Jarir Atthobari 1^e
Drug effects on albuminuria and renal function
10. 2003-2006 Christiane A Geluk industry
Myocardial ischemia in asymptomatic individuals
11. 2004-2006 Auke H Brantsma 1^e
Development and consequences of increased levels of UAE and CRP
12. 2004-2007 Riko HH Nap NSN/1^e
Volume homeostasis in microalbuminuria
13. 2004-2007 Jacoline W Brinkman industry
Laboratory methods for cardiovascular and renal risk markers
14. 2005-2008 Nynke Halbesma NSN
Progressive renal function loss in subjects not known with a renal disease
15. 2005-2007 Cornelis Boersma NSN
Cost-effectiveness of screening for microalbuminuria
16. 2005-2007 Yvonne de Kluzenaar TNO
The combined influence of noise and air pollution on health
17. 2005-2008 Akin Ozyilmaz 1^e
Screening for renal damage to improve cardiovascular outcome
18. 2005-2007 Annemieke Roos 1^e
Thyroid function in relation to CV disease
19. 2006-2008 Anne Chris Jonkers 1^e
Simple risk markers for cardiovascular disease in the general population

PREVEND IN THE INTERNATIONAL LITERATURE

Citations of the PREVEND studies

To date 16 research fellows are involved or have been involved in generating the scientific output. While in the initial years two fellows were appointed, the number of PhD students increases gradually (figure 8). Both funding from the Faculty of Medicine and of the Faculty of Sciences and Mathematics are involved, as well as funding from the Dutch Kidney Foundation (NSN), the Dutch Heart Foundation (NHS), various pharmaceutical (Bristol Myers and Squibb), and laboratory diagnostic companies (Dade Behring, Abbott, Ausam).

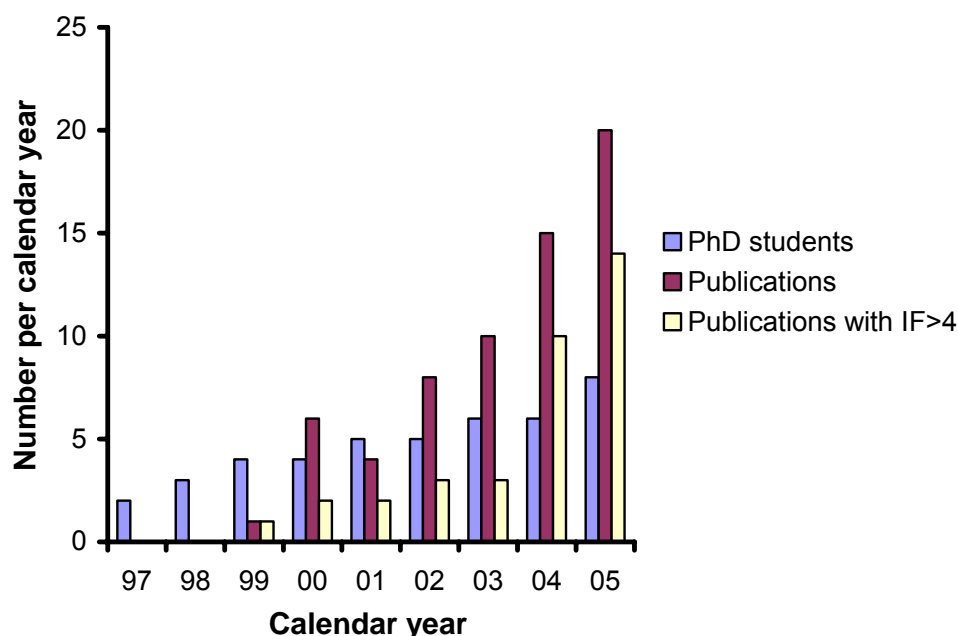


Figure 8. *Number of PhD students working with the PREVEND database and their output.*

The PREVEND scientific output in the report of the first survey counted about 5 papers per year, while it since then steadily increased to more than 15 papers in 2004 (figure 9). Initially about one third was published in journals with an impact factor of more than 4. Presently more than two thirds of the papers are published in journals with an impact factor above 4 (figure 8).

As per July 2005 the first 30 papers published by the PREVEND study group were cited already more than 500 times. The paper by Hillege on the predictive value of albuminuria for mortality (ref 17) was cited more than 100 times. The papers by Hillege on the prevalence of microalbuminuria in the general population (ref 9) and by Pinto-Sietsma on the impact of smoking on albuminuria (ref 5) and the relation between glomerular hyperfiltration and albuminuria (ref 6) had more than 50 citations. This emphasises that not only the societal impact of the study, but also the pathophysiological concepts that are brought forward from these epidemiological data, deserve much interest.

Editorials or Commentaries on PREVEND data

This conclusion is also clear from the editorials and commentaries that have been published in combination with some of the reports.

In an editorial, entitled “Vive la petite difference” accompanying the paper by Verhave on the different sensitivity of men and women for the influence of CV risk factors, Ritz wrote “this data of the PREVEND study provides welcome novel information. As all good studies, the present report raises more questions than it answers, but it will certainly not fail to stimulate future research in this still murky and unresolved area of cardiovascular research” (i).

In a commentary on the paper by Hillege on the predictive value of albuminuria on CV and non-CV mortality (ref 17) Hollenberg wrote “PREVEND is a heroic study. The investigators attempted to enroll the entire adult population of Groningen and almost half responded and were included”. “One wonders whether measure of urinary albumin might identify folks in whom treatment with ACEi or ARB’s is appropriate, even in the absence of hypertension or diabetes. Perhaps the citizens of Groningen can help us to answer that question”(ii).

An editorial accompanied the paper by Verhave on the influence of sodium intake on albuminuria (ref 37). It stated “it is the merit of this study to put squarely back onto the map the hotly debated issue that had caused considerable controversy in the past, i.e. the impact of sodium intake in hypertensive and renal patients” (iii). “This paper by Verhave et al will hopefully serve to wake up the renal community: there may be a chance out there to improve renal outcomes”

A commentary on the publication of the PREVEND intervention trial by Asselbergs (ref 40) was entitled: “Treating microalbuminuria: cosmetic exercises with urine chemistry or effective reduction of clinical events?” The editor argued that “it is not easy to find a suitable cohort of patients for such a trial, but the PREVEND study provided a unique opportunity for this endeavour” (iv). He concluded that the study raises the hope that RAAS blockade provides benefits beyond BP lowering, even in low-risk individuals presenting with nothing but urinary albumin values in the high normal or microalbuminuric range. The public health importance of this issue is obvious”.

PREVEND reviews, Invited lectures and ISN endorsed symposia

The PREVEND study group has been invited to write various reviews to describe the possibilities of setting up screening programs involving the measurement of albuminuria to detect and treat subjects at risk for ESRD (57,58,63-65,76,77).

PREVEND researchers have given invited lectures at the ASN in 2004 in St Louis, at the EDTA/ERA in 2005 in Istanbul, at the ISN in Singapore in 2005 and in ISN endorsed symposia in Johannesburg in 2003, Bellagio in 2004, Hong Kong in 2004 and Oman in 2005. It indicates that the PREVEND program is considered an example on how to set up prevention programs for renal disease.

The widespread belief that albuminuria should be considered as an early test for renal and vascular disease was also the conclusion of an ISN-endorsed meeting organised by the PREVEND investigators in Amsterdam November 2004. Remuzzi wrote on it in an editorial “A consensus of that meeting was presented to the Chief Medical Officer of Europe, Herre Kingma, who promised to take this further to his European colleagues. Kingma is considering to complement the health-screening centres for infants and young children with a similar function for adults aimed at early detection and prevention of chronic diseases, for which measurement of albuminuria could be one of the variables to monitor”(v)

- i. Ritz E. Cardiovascular risk factors and urinary albumin: vive la petite difference. An Editorial on: Verhave JC et al. Cardiovascular risk factors are differently associated with urinary albumin excretion in men and women. *J Am Soc Nephrol* 2003; 14: 1415-1416
- ii. Hollenberg NK. A commentary on: Hillege et al. Urinary albumin excretion predicts cardiovascular and non-cardiovascular mortality in the general population. *Curr Hypertens Rep* 2003; 5: 356-357
- iii. Ritz E. Salt-The forgotten Renal Risk Factor. A commentary on: Verhave JC et al. Sodium intake affects urinary albumin excretion especially in overweight subjects. *J Am Soc Nephrol* 2005; 16: 567-573
- iv. Ritz E. Treating microalbuminuria – Cosmetic exercises with urine chemistry or effective reduction of Clinical Events. A commentary on Asselbergs et al. Effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria. *J Am Soc Nephrol* 2005; 16:1159-1163.
- v. Remuzzi G, Weening JJ. Albuminuria as early test for vascular disease. *Lancet* 2005; 365:556-557

LOGISTICS

CREATING THE PREVEND COHORTS

The study started in 1997 by inviting all Groningen inhabitants aged 28-75 years to send in a morning urine sample and to answer a short questionnaire. Of the 85,421 subjects invited 40,856 responded (=48%) (see for description ref 9). We invited all consenting subjects with a morning urinary albumin concentration (UAC) of >10 mg/L (n=7,768) and an a-select sample of those with an UAC <10 mg/L (n=3,395) for further studies (see for description of the methods ref 6). Of these invitees 8,592 subjects completed the first screening, that was held in 1997/1998 (figure 1). This is the PREVEND cohort, which by purpose is enriched for the presence of an elevated urinary albumin excretion (UAE). For a schematic representation how the PREVEND cohort was formed we refer to figure 1.

Because enrichment for albuminuria may influence some of our data analyses, we also wanted to study a cohort that is a representative sample of the Groningen population. To that purpose we took all subjects with an UAC <10 mg/L that completed the first screening (N=2,592) and added a subset of the "oversampled" subjects with an UAC >10mg/L by proportionally taking a SPSS generated random subset (n=840). This resulted in a group of 3,432 subjects (see for description ref 46)

FOLLOW UP OF THE PREVEND COHORTS

The city Groningen cohort (n=40,856)

Since 1997 the large cohort of 40,856 subjects is followed just for survival. To that purpose the data of the death certificates of the Central Bureau for Statistics are linked yearly to the PREVEND database (see for description ref 17).

The PREVEND cohort (n=8,592)

From 1997 on the death certificates of the PREVEND cohort of 8,592 subjects are thus also available. Of these subjects the clinical events (not leading to death) are also registered using the Dutch PRISMANT database of hospital discharge diagnoses (see for description ref 72). In addition, these subjects are seen every 3-4 year on the out-patient PREVEND facility.

The second screening took place from 2001-2003 (mean follow up versus first screening 4.2 years), the third one is running from 2003-2006 (mean follow up versus first screening about 6.6 years). Presently we are preparing the fourth screening. The second screening was completed by 6,894 subjects. Of the 8,592 original subjects 240 had died (0.7% per year), and 1,458 withdrew consent for further screening, resulting in a withdrawal rate of 4.0% per year. It is to expect that approximately 6,000 subjects will complete the third screening and that approximately 400 subjects will have died before the end of the third screening.

In the second and third screening the same data are collected as in the first screening (figure 1). In addition at these latter screenings a PortaPres measurement and a body-impedance-measurement have been performed, and subjects also answered a questionnaire on neuroticism (the Eysenck Personality Questionnaire Revised) and on somatic and psychiatric ill-health (the General Health Questionnaire) (see ref 76). In the third screening cognitive functioning is being measured using the RFFT (Ruff Figural Fluency Test) (Berning et al, Assessment 1998;5:181-186, Ross et al, Arch Clin Neuropsychol 2003;18:879-891) and mild anterograde amnesia as early sign of developing dementia of the Alzheimer type using the VAT (Visual Association Test) (Lindeboom et al, J Neurol Neurosurg Psychiatr 2002;73:126-133).

The SALUT cohort

Part of the subjects participating in the PREVEND study have at the time of the second PREVEND screening also been invited to participate in the SALUT (Study of Allostatic Load as a Unifying Theme) study. This study aims to examine the extent to which propensity to ill-health of any kind, including psychiatric and traumatic, is attributable to liability, susceptibility or chains of risk models of morbidity accumulation. The SALUT participants underwent an intelligence test and a structured psychiatric interview assessing somatization, anxiety and depression. In addition, they completed a self-administered questionnaire covering physical and mental health, stress, personality, social support, lifestyle factors and demographic characteristics. These measurements are repeated every three years.

The PREVEND IT cohort (n=864)

From the actual PREVEND cohort of 8,592 subjects all subjects with an UAE of 15-300 mg per day, a blood pressure <160/100 mmHg and a plasma cholesterol level of <8.0 mmol/L (<5.0 in case of previous myocardial infarction) were invited to participate in the PREVEND Intervention Trial (PREVEND-IT). Altogether 964 subjects were included. The study (for description see ref 3) lasted four years. At that time the trial drugs were stopped, and the patients are followed again in the actual PREVEND cohort

The Groningen random sample (n=3,432)

As these patients are part of the actual PREVEND cohort, the data as collected in the actual PREVEND cohort are also available in this random Groningen sample.

THE PREVEND STUDY GROUP

Data as of July 2005. See for research fellows separate list at page 19

Groups in Groningen

| | |
|---|---|
| <i>Cardiology</i> | Hillege HL Van Veldhuisen DJ |
| <i>Clinical Epidemiology</i> | Van Voors AA Burgerhof JGM Stolk RP |
| <i>Clinical Pharmacology</i> | De Zeeuw D Van Gilst WH |
| <i>Endocrinology</i> | Wolffenbuttel BHR Dullaart RPF |
| <i>Geriatric Medicine</i> | Slaets JPJ Izaks G |
| <i>Internal Medicine</i> | Bakker SJL Gans ROB |
| <i>Medical Genetics</i> | Te Meerman GJ Van der Steege G Korstanje R Zuurman MW |
| <i>Medical Physiology</i> | Muntinga JHJ |
| <i>Nephrology</i> | De Jong PE Gansevoort RT Navis GJ |
| <i>Pharmacoepidemiology and Pharmacoeconomics</i> | De Jong-van den Berg LT Postma MJ V.d. Berg PB Visser ST |
| <i>Social Psychiatry</i> | Neeleman J Rosmalen JGM |
| <i>Vascular Medicine</i> | Smit AJ Van Roon AM |
| Supporting Staff | |
| <i>Secretariat</i> | De Jonge-Hovenkamp DGA Graler MJS |
| <i>Research nurses</i> | Bos-Bolt A Bronger AA Dijkstra-Oppenhuisen R Klijn-de Winter A Van Zanten M |
| <i>Supervising nurse</i> | Groenewegen NA |
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| <i>Laboratory Management</i> | Bakker SJL de Zeeuw D Duker JJ Gansevoort RT |

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Bieringa RJ
Hillege HL
Steenks DH
Van der Velde O

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Environment and Health, TNO, Delft
Vascular Medicine, AMC, Amsterdam
Epidemiology/Public Health, Harvard, Boston, MA
Computational Genetics, Dartmouth, Lebanon, NH
Medicine, Yale, New Haven, CT
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Miedema HME
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Curhan GC
Moore JH
Hebert PR
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2. The Netherlands Organization of Scientific Research for genetic studies on polymorphisms of the Renin Angiotensin System (901-03-153)
3. The Netherlands Organization of Scientific Research for studies on intima media thickness and flow mediated dilatation (902-18-319)
4. The Netherlands Organization of Scientific Research for the Study of Allostatic Load as a Unifying Theme (900-00-02)
5. The Netherlands Organization of Scientific Research for studies on the determinants of HPA axis activity (016.056.064)
6. National Institute of Health for studies on tissue Plasminogen Activator and Plasminogen Activator Inhibitor
7. Ministry of Environmental Affairs for studies on effects of noise and air pollution on health status
8. The Dutch Kidney Foundation for studies on volume homeostasis (C01.1969)
9. The Dutch Kidney Foundation for studies on mechanisms of progressive renal function loss (C04.2091)
10. The Dutch Kidney Foundation for studies on cost-effectiveness of screening and treatment of microalbuminuria (PV 11)
11. The Dutch Heart Foundation for electrocardiographic studies in microalbuminuria (1999.103)
12. The Dutch Heart Foundation for studies on lipid metabolism (2001-005)
13. The Dutch Heart Foundation for studies on genetic polymorphisms in relation to vascular function
14. De Cock Foundation for studies on urinary electrolytes in relation to microalbuminuria
15. De Cock Foundation for studies on CMV in relation to vascular damage
16. De Cock Foundation for studies on Apolipoprotein All in relation to vascular damage

DATA COLLECTED IN PREVEND

| | City cohort | PREVEND cohort | | | Random sample |
|--|--------------------|-----------------------|-------|-------|----------------------|
| Year | 97/98 | 97/98 | 01/02 | 03/04 | Idem |
| Number | 40,856 | 8,592 | 6,894 | | 3,432 |
| Various measurements and anthropometry | | | | | |
| Short questionnaire | X | X | | | X |
| Extended questionnaire | | X | X | X | X |
| Anthropometry | | X | X | X | X |
| Blood Pressure Dynamap | | X | X | X | X |
| Ankle Brachial Index | | X | X | X | X |
| Portapres registration | | | X | X | |
| Body impedance | | | X | X | |
| ECG | | X | X | X | X |
| Morning urine sample | X | X | | | X |
| Two 24 hr urines | | X | X | X | X |
| Blood chemistry | | X | X | X | X |
| DNA genotyping | | X | | | X |
| Cognition tests | | | | X | |
| CBS death certificates | | X | X | X | X |
| Hospital discharge diagnoses | | X | X | X | X |
| Biochemistry parameters per 1-9-2005 (tbd = to be done) | | | | | |
| Blood | | | | | |
| Hb | | X | | X | X |
| Creatinine | | X | X | X | X |
| Total cholesterol | | X | X | X | X |
| HDL cholesterol | | X | Tbd | X | X |
| Glucose | | X | X | X | X |
| Triglycerides | | X | Tbd | | X |
| CRP | | X | Tbd | | X |
| ApoI | | X | | | X |
| ApoAll | | X | | | X |
| ApoB | | X | | | X |
| Lp(a) | | X | | | X |
| Von Willebrand factor | | X | Tbd | | X |
| Cystatin C | | X | Tbd | | X |
| Insulin | | X | | | X |
| ProBNP | | X | Tbd | | X |
| Cortisol | | X | | | X |
| Uric acid | | X | | | X |
| TSH | | X | | | X |
| FT4 | | X | | | X |
| FT3 | | X | | | X |
| Anti-TPO | | X | | | X |
| IgG ant-CMV | | X | | | X |
| PAI-1 | | X | | | X |
| TPA | | X | | | X |
| Urine | | | | | |
| Albumin | X | X | X | X | X |
| Creatinine | X | X | X | x | X |
| Sodium | | X | X | | X |
| Potassium | | X | X | | X |
| Uric acid | | X | X | | X |
| Calcium | | X | X | | X |
| Urea | | X | X | | X |
| Chloride | | | X | | X |