Higher glomerular filtration rate as a risk factor for loss of kidney function in early type 2 diabetes and the general population

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Rationale & Objective: An abnormally elevated glomerular filtration rate (GFR), or renal hyperfiltration, may predispose individuals to subsequent rapid GFR decline in diabetes, obesity and the metabolic syndrome. Although this hypothesis is supported by results of experimental studies, the importance of hyperfiltration at the population level remains controversial. We investigated whether a higher baseline GFR predicts a steeper medium- to long-term GFR decline in type 2 diabetes and in a general population without diabetes.

Study Design: Longitudinal cohort studies.

Setting & Participants: 319 Pima Indians, of whom 83% had type 2 diabetes, and 1594 middle-aged non-diabetic Caucasians (the Renal Iohexol Clearance Survey [RENIS]).

Predictor: Baseline measured GFR using exogenous clearance methods.

Outcomes: GFR decline rates.

Analytical Approach: Because spurious correlations between initial values (e.g., GFR level) and subsequent changes may bias ordinary regression methods, we used a linear mixed model to assess the correlation between baseline GFR (the random intercept) and GFR decline rate (random slope). This method separately estimates the error term (e.g., the day-to-day variation in the GFR) and random effects, minimizing bias because of regression to the mean.

Results: The mean (SD) baseline GFRs were 149.4 (43.3) and 104.0 (20.1) ml/min, and the median (IQR) follow-up were 9.1 (4.0-15.0) and 5.6 (5.2–6.0) years in the Pima and RENIS cohorts, respectively. The correlation between baseline GFR (the random intercept) and GFR decline rate (slope) was -0.41 (95% CI -0.55, -0.26) in the Pima cohort and -0.31 (-0.40, -0.23) in the RENIS cohort, adjusted for age, sex, height and weight, demonstrating that higher baseline GFR values were associated with steeper GFR decline rates.

Limitations: Different methods for measuring the GFR in the two cohorts.

Conclusions: A higher baseline GFR is a potentially modifiable risk factor for medium- to long-term GFR decline in type 2 diabetes and the general population.

Nontechnical summary

Abnormally elevated kidney filtration, or hyperfiltration, causes kidney damage in animal experiments. Elevated kidney filtration is common in persons with prediabetes, obesity and diabetes, but it is unclear if it leads to loss of kidney function in humans.

We measured kidney function in two longitudinal studies; 319 Native Americans in Arizona, most of whom had type 2 diabetes, and 1594 middle-aged non-diabetic Norwegians. In both studies we found that individuals with higher kidney filtration had an increased risk for subsequent loss of kidney function during 6-9 years of follow-up.

Elevated kidney filtration may be a target for early preventive measures to reduce kidney function loss in persons at risk of chronic kidney disease.

Index words: Glomerular filtration rate (GFR), renal hyperfiltration, glomerular hyperfiltration, type 2 diabetes, prediabetes, general population, non-diabetic, GFR decline, kidney function, Native Americans, measured GFR, iohexol clearance, iothalamate clearance, regression to the mean.

Chronic kidney disease (CKD) is a global health problem affecting 10-15% of all adults, and 15-45% of people aged 65-74 years old.[1] The death rate from CKD has increased by approximately 40% since 1990 in Western countries, and the number of patients receiving dialysis has increased rapidly worldwide.[2]

Age-related loss of the glomerular filtration rate (GFR) is the most important predisposing cause of CKD [3,4]. However, while some people experience a rapid decline in GFR with age, others retain a relatively well-preserved GFR.[3,4] The different rates of GFR decline between individuals is poorly understood and only partially explained by risk factors such as diabetes and hypertension.[1,3,5]

Studies in animals found that an elevated single nephron GFR (the filtration rate in the smallest functional unit of the kidney), or glomerular hyperfiltration, mediates progressive kidney disease caused by a variety of initiating injuries, including partial nephrectomy (surgical removal of part of the kidney).[6] Abnormally elevated whole kidney GFR, or renal hyperfiltration, affects 20-70% of patients with diabetes.[7] Increasing evidence supports that renal hyperfiltration predicts the initiation of diabetic kidney disease, although the role of hyperfiltration as an independent risk factor for long-term loss of GFR remains controversial, particularly in type 2 diabetes.[7,8] Renal hyperfiltration may also affect a considerable proportion of the general non-diabetic population, in which higher GFR levels have been linked to prediabetes, obesity, tobacco smoking, the metabolic syndrome and hypertension.[9-11] However, whether a higher GFR is a risk factor for an accelerated GFR decline has not been investigated in the general non-diabetic population.

Renal hyperfiltration is difficult to study at a population level because statistical analyses relating change to the baseline value using ordinary linear regression or correlation are hampered by mathematical coupling and regression to the mean.[12,13] Moreover, using an arbitrary whole kidney GFR cut-off (for example >140 ml/min) to define hyperfiltration is

problematic, particularly in middle-aged and elderly persons, because the number of nephrons declines with age and varies significantly between individuals.[7,14] Glomerular hyperfiltration (at the single nephron level) may therefore occur throughout the range of whole kidney GFR, because whole kidney GFR = single nephron GFR x nephron number.[14-16] Finally, the commonly used creatinine- and cystatin C-based estimates of GFR lack precision and are biased in the high-normal range of GFR.[10,17]

In this study, we addressed these methodological problems by using the measured GFR and linear mixed model regression analyses to address the problem of regression to the mean (see Methods and Discussion).[12,13] To explore whether a higher GFR is a common risk factor associated with rapid GFR decline in the presence or absence of diabetes, we investigated two diverse cohorts, a cohort of Pima Indians from Arizona, USA, and a representative sample of middle-aged non-diabetic Norwegians with a high prevalence of prediabetes and metabolic syndrome.[18,19] Most of the Pima Indians had type 2 diabetes, since they were part of a study examining the hemodynamic effects of type 2 diabetes on the kidneys.[20] We hypothesized that a higher baseline measured GFR is associated with a steeper GFR decline regardless of the presence or absence of diabetes.

METHODS

Study Participants

The Pima Indians in the study were recruited from the Gila River Indian Community in Arizona as part of the Diabetic Renal Disease Study (DRDS) and the "Renoprotection in Early Diabetic Nephropathy in Pima Indians" trial (ClinicalTrials.gov number, NCT00340678; "the Losartan trial").[20,21] The DRDS (1989-1994) consisted of 194 people with different stages of glycemia. The GFR was measured annually or every 6 months for 2-4

years. A subset of 51 people was followed with GFR measurements for an additional two years in a subsequent protocol. The Losartan trial (1996-2001) included 169 Pima Indians with type 2 diabetes and normo-albuminuria or micro-albuminuria who were randomized to receive losartan treatment or placebo; they were followed for 6 years with annual GFR measurements.[21] Following trial completion, participants continued to be followed with annual GFR measurements. In the current study, we included all 319 Pima Indians with GFR measurements who were included in the DRDS and/or Losartan trial. Each participant was included once only, and their earliest examination at which GFR was measured in either the DRDS or Losartan trial was selected.

The Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6) was conducted in 2007-2009 as a sub-study of the population-based sixth Tromsø study (Tromsø 6) in the municipality of Tromsø, northern Norway.[22] A random sample of Tromsø's inhabitants aged 50- 62 years was invited to participate (N = 5464 persons). A total of 3564 (65%) completed the Tromsø 6 study, and from these, we invited participants who did not report cardiovascular disease, kidney disease or diabetes (N = 2825) to RENIS-T6. From the 2107 (74%) who gave a positive response, we included 1632 persons in consecutive order according to a predetermined target size. A description of the study participants and enrollment in RENIS-T6 was published previously.[22] Five participants had a technical failure in the GFR measurement and 33 had diabetes at baseline according to their fasting plasma samples or HbA1c. These 38 were excluded from the current study, leaving 1594 participants for inclusion, of whom 30 percent had the metabolic syndrome and 47 percent prediabetes (fasting glucose 5.6-6.9 mmol/L and/or HbA1c 5.7-6.4%; according to American Diabetes Association), as reported in two previous publications from RENIS.[18,19] A total of 1299 (81%) participants had a follow-up examination between 2013 and 2015 (Figure S1).[5]

The RENIS study was approved by the Committee for Health Research Ethics of North Norway and the Pima studies were approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. The studies adhered to the Declaration of Helsinki. All study participants gave informed consent.

Data

We measured the GFR after an overnight fast using single-sample plasma clearance of iohexol in the RENIS cohort and by the urinary clearance of iothalamate in the Pima cohort.[22,23] Both methods have been validated as accurate methods,[17,24] and are described in Item S1.[17,24] Details regarding health questionnaires and other measurements are reported elsewhere.[9,20,22,25]

Statistical Analysis

The GFR measurements were analyzed in a linear mixed regression model with a random intercept and slope using an unstructured covariance matrix.[26,27] Details are presented in Item S1. The absolute GFR measured in ml/min (at each visit) was used as the dependent variable. The observation time from baseline was used as the independent time variable. The effect of an independent variable on the GFR change rate in ml/min/year (slope) was analyzed by including two-way interaction terms between the independent variable in question and the time variable. The association between the baseline GFR and subsequent GFR change rate (ml/min/year) was assessed by the correlation between the random intercept and random slope.[13,27] We adjusted for the following covariates: age and sex in model 1; age, sex, height and weight in model 2; and age, sex, height, weight, blood pressure (BP), fasting glucose, the use of antihypertensive medications, urinary albumin-to-creatinine ratio (ACR) and current smoking status (the latter variable only available in RENIS) in model 3. In model 3 for the

Pima cohort, we also included the diabetes duration, study protocol and use of insulin or oral antidiabetic medications.

Statistical significance was set at 0.05. All statistical analyses were performed in Stata/MP 14.0 (www.stata.com).

RESULTS

Study population characteristics

Table 1 shows the baseline characteristics of the two study populations. Eighty-three percent (N=266) of the Pima Indians had diabetes at baseline, and their mean BMI was 34.8 (8.2) compared to 27.2 (4.0) in the RENIS cohort. The mean (SD) baseline GFR (not adjusted for body surface area (BSA)) was 149.4 (43.3) ml/min (range 48 - 296 ml/min) in the Pima cohort and 103.8 (19.9) ml/min (range 24.2 – 140.9 ml/min) in the RENIS cohort. The median follow-up was 9.1 (IQR; 4.0-15.0) years in the Pima cohort and 5.6 years (IQR; 5.2–6.0) in the RENIS-FU. The unadjusted GFR decline rates were 3.60 (5.35) ml/min/year and 0.95 (2.23) ml/min/year in the two cohorts respectively.

Association of baseline characteristics with the baseline GFR and GFR change rates

Lower age, higher body weight and higher fasting plasma glucose concentration were associated with a higher baseline GFR in both cohorts in multivariable adjusted linear mixed regression models with a random intercept and slope (Tables 2 and 3). Male sex was associated with a higher baseline GFR in the RENIS study but not in the Pima cohort. The fasting plasma glucose concentration, ACR and diabetes duration were associated with a steeper rate of GFR decline in the Pima cohort, and age, smoking, and ACR were associated with a steeper rate of GFR decline in RENIS. The effect of smoking and ACR in RENIS was moderate and borderline statistically significant (P=0.05)

Association between the baseline GFR and GFR change rate

There was a negative correlation between the GFR at baseline (random intercept) and the GFR change rate (random slope) in both cohorts (Tables 2 and 3). The negative correlation persisted after adjusting for height and weight, and in the fully adjusted model (Tables 2 and 3). This demonstrates that higher baseline GFR values (random intercepts) were associated with steeper GFR decline (random slopes). The correlation implies that a 10 mL/min higher baseline GFR is associated with an annual rate of change that is 0.31 mL/min/year faster in the RENIS cohort and 0.46 mL/min/year faster in the Pima cohort, in the model adjusted for age, sex, height and weight (Table 2 and 3).

The correlation between baseline GFR and GFR change rate was essentially the same, and significant in all models, when we adjusted for HbA1c instead of fasting glucose, systolic BP instead of diastolic BP, and for change in antihypertensive medications during follow-up. The negative correlation between baseline GFR and GFR change rates was similar for both genders in RENIS, but it was only significant for women in the Pima cohort (p<0.001 for interaction) (Table S1).

To study Pima Indians with type 2 diabetes separately, we excluded those 53 (17%) without diabetes at baseline; the results were similar (r=-0.37 [95% CI -0.52 to -0.20] in model 2).

Sensitivity analyses

Although persons with acute illness or self reported kidney disease were excluded from the RENIS study, 30 persons had baseline GFR < 60 ml/min/1.73 m^2. We repeated the analyses after excluding persons with baseline GFR < 60 ml/min/1.73 m^2 in both cohorts [n=3 in the Pima cohort and n=30 in RENIS]). The correlation between baseline GFR (the random intercept) and GFR change rate (random slope) remained significant for both cohorts, as shown in Figure 1. We performed separate analyses for the 169 Pima Indians who were included in the Losartan trial (all with diabetes) and the remaining 150 who were not included

(53 of whom did not have diabetes). The negative adjusted correlation between the baseline GFR and GFR change rate (the intercept and slope) was significant in both groups (r=-0.52 [95% CI -0.65, -0.35] vs r = -0.31 [95% CI -0.49, -0.10]), respectively. The study treatment did not affect the relationship between baseline GFR and GFR change rate for those in the Losartan clinical trial.

To assess the correlation between the random intercept and slope using the relative decrease in GFR, we used the log-transformed GFR as the dependent variable. The correlation between the random intercept and slope was attenuated and only statistically significant for women in both cohorts (Table S2). Inspection of the residuals in these models revealed a slightly asymmetric distribution, suggesting that these results should be interpreted with caution. Finally, we repeated the analyses using GFR standardized to body-surface-area (ml/min/1.73 m^2). The correlation between baseline GFR and GFR change rate were essentially the same (Pima model 2: r=-0.42 [95% CI -0.56, -0.26], RENIS model 2: (r=-0.33 [95% CI -0.41, -0.25]).

DISCUSSION

GFR was higher in the predominantly diabetic Pima Indian cohort than in the non-diabetic general population cohort, and the average rate of GFR decline was greater. Nevertheless, despite their differences, higher baseline GFR was associated with a subsequent steeper GFR decline in either cohort, suggesting that higher GFR is associated with a faster medium- to long-term GFR decline, regardless of the presence or absence of diabetes.

A few previous studies investigated the association between the baseline GFR, or baseline hyperfiltration status, and subsequent GFR decline. In a population-based outpatient dataset (N=1,526,437), including 6% with diabetes, Tonelli et al found that a higher estimated GFR (eGFR) was associated with an increased risk of doubling in serum creatinine during a median

follow-up of 35 months.[28] Two other studies found that higher baseline eGFR levels predicted a steeper eGFR decline in large community cohorts using mixed model analyses.[29,30] However, they did not report the correlation between the random intercept and slope, and they examined the baseline eGFR as a categorical fixed effect variable. Using a similar method, a recent study found that a higher baseline measured GFR was associated with a more rapid decline in GFR in patients with type 1 diabetes during a median follow-up of 19 years.[31] Finally, hyperfiltration, defined as the estimated or measured GFR above a certain threshold (ranging from 120–137 ml/min/1.73 m^2), was associated with an increased risk of rapid GFR decline in some, although not all, studies in type 1 and type 2 diabetes that used either traditional logistic or linear regression analyses.[7,32]

Although these studies seem to suggest that higher GFR levels increase the risk for subsequent accelerated GFR decline, none of the studies addressed the statistical phenomenon of regression to the mean. This means that high observations are likely to be followed by less extreme ones nearer the subject's true mean. This statistical phenomenon may result in a spurious relationship between baseline GFR and GFR decline when analyzed with conventional regression models.[12,13] We used the method of Blance et al., who suggested a 2-level linear mixed model as a solution to the problem.[13] Unlike an ordinary linear regression model, this model separately estimates the variation in the GFR and residual error (the measurement error and biological day-to-day variation in the GFR measurement). The correlation between the baseline GFR and GFR change rate can then be tested without the influence of the error term that mediates the problem of regression to the mean. In the present study, we used this method in both a small cohort with many GFR measurements and in a larger cohort with the minimum GFR measurements necessary to estimate the variance components. The correlations between the intercept and slope were similar in the two cohorts,

which suggests that higher GFR levels, indicating hyperfiltration in at least a proportion of the participants, may play a role in accelerated GFR decline in both cohorts.

The negative correlation between the initial GFR and subsequent rate of GFR change also implies that people with a GFR lower than the mean have a slower GFR decline, as shown in Figure 1. This may represent minimal decline in subjects with optimal hemodynamic conditions at the glomerular level, but we cannot exclude that some individuals may be recovering from an episode of reduced GFR due to illness or acute kidney injury. However, individuals with acute illness did not have their GFR measured during the illness, and we obtained similar results after excluding those with a reduced GFR at baseline.

We used absolute GFR (ml/min) in the main analyses because the practice of indexing GFR for body surface will obscure (underdiagnose) hyperfiltration in obesity.[33] However, separate analyses using indexed GFR (ml/min/1.73 m2) yielded similar results.

We investigated the issue of renal hyperfiltration without a fixed cutoff GFR to define hyperfiltration, e.g GFR>140 ml/min. A high-normal age-adjusted whole kidney GFR may also represent glomerular hyperfiltration, because there is considerable variation in the number of nephrons with age and between individuals.[14] For example, a GFR of 80 ml/min/1.73 m^2 would likely reflect glomerular hyperfiltration in a person with 50% reduction in nephron number. In healthy kidney donors, Denic et al. found that the numbers of nephrons decreased linearly from the age of 20 years to approximately 50% at the age of 70.[14] Another study of healthy kidney donors reported that high age-adjusted measured GFR was associated with higher single nephron GFR, higher 24-h urine albumin, and larger glomerular volume.[34] Although nephron number decrease with age, GFR increased during a time period of 3-6 years in approximately 30% of non-diabetic adults from the general population in other studies,[15,35,36] indicating an increased single nephron GFR in many people. Moreover, a longitudinal increase in the GFR was independently associated with increasing albuminuria in

the RENIS study, indicating possible early alterations in the filtration barrier and/or decreased reabsorption of albumin in the hyperfiltration state.[15]

Previous studies linking higher fasting glucose, body weight and waist-to-hip ratio to higher measured GFR within the normal range of GFR (in linear regression with mGFR as a continuous dependent variable) also suggests that single nephron hyperfiltration occurs through the range of GFR.[9,37] In type 2 diabetes, an acute reduction of the GFR within the near-normal range of GFR was inversely associated with the subsequent eGFR change rates in both the RENAAL and EMPA-REG trials, suggesting a possible protective effect of treating glomerular hyperfiltration within the normal range of GFR.[38,39]

In a previous investigation of a subsample from the current Pima cohort consisting of 111 persons with protocol kidney biopsies, Fufaa et al reported higher GFRs in people with glomerular hypertrophy and increased podocyte foot process width.[25] These histological correlates of glomerular hyperfiltration were also associated with an increased risk of rapid GFR decline at follow-up.[25]

The negative correlation between the baseline GFR and GFR decline in the Pima cohort was significant for women only. There were fewer men (n=109) compared to women (n=210) in the Pima cohort and the sex-interaction should be interpreted with caution. However, we note that the protective effect of female sex on CKD progression among non-diabetic individuals may be attenuated in diabetes.[40] A recent study found that women with diabetes and hyperfiltration had a higher glomerular pressure than men with diabetes and hyperfiltration, which possibly indicates a more pathologic subgroup of hyperfiltration.[6,41]

There are limitations of this study. Because whole kidney GFR is a product of nephron number and single nephron GFR we cannot conclude that a higher GFR reflects higher single nephron GFR. Indeed, people with low GFR and severely reduced nephron number are often

hyperfiltering, but this cannot be detected by measuring whole kidney GFR. However, to a certain degree we accounted for this problem by adjusting for factors that are associated with nephron number (age, sex and height).

We used different methods for measuring the GFR in the two cohorts, although both accurately reflected the gold standard method of urinary inulin clearance.[17,24] The mixed model analyses necessarily assume a linear decline in GFR, which is not true for many participants. Still, this is probably a good approximation of the medium-term change in the GFR for most individuals.[42] Although we accounted for the problem of regression to the mean, we acknowledge that a negative correlation between the intercepts and slopes may still arise from natural biological variation even if a high baseline GFR is not the cause of the more rapid long-term GFR decline in these subjects.

Most participants in the current study still had a GFR within the normal range at follow-up. Accordingly, we cannot conclude that those with a higher GFR and steeper decline subsequently progress to CKD or kidney failure. However, a rapid loss of GFR within the normal range of GFR is still potentially clinically important because it is associated with an increased risk of end-stage renal disease (ERSD) and all-cause mortality in both high- and low-risk groups of the general population.[35,36,42] Indeed, accelerated early GFR loss has been identified as a phenotype with increased risk of ESRD in the Joslin diabetes cohort.[43]

Strengths of this study include obtaining equivalent results in two very different cohorts, suggesting broad generalizability of our findings. In addition, GFR measurements were performed with accurate clearance methods. The RENIS cohort is the only longitudinal study with repeated measurements of the GFR in a representative sample of the non-diabetic general population. The intra-individual day-to-day variation in the GFR measurement in the RENIS cohort was lower than in most previous studies.[5,17] In the Pima cohort, we measured the GFR multiple times over an extended period in people with a high risk of CKD and ESRD. The

day-to-day variation in the GFR measurement using urinary iothalamate clearance was 5-15 ml/min in different studies,[5,17] although it was not calculated in the Pima cohort. Finally, we used a linear mixed regression model that handles the problem of mathematical coupling and regression to the mean.

We conclude that higher baseline GFRs are associated with a steeper GFR decline in the general non-diabetic white population and in Native Americans with prevalent T2D. This study supports the belief that higher GFR levels, indicating hyperfiltration in at least a proportion of individuals, is a potential modifiable risk factor for loss of kidney function in diverse populations.

Acknowledgments

Supplementary Material

Supplementary Item S1
Supplementary methods and statistics

Supplementary Figure S1
Inclusion of participants in the RENIS Follow-Up Study

Supplementary Table S1

Sex specific random effects of a mixed linear regression in the Pima cohort.

Supplementary Table S2

Sex specific random effects of a mixed linear regression model using log-transformed GFR

Figure Legends

Figure 1. The association between baseline GFR and GFR change rate

The figure displays the best linear unbiased predictors (BLUPs) of the random slopes and intercepts, reflecting the association between baseline GFR (the random intercept) and the GFR change rate (random slope) in a linear mixed model after excluding people with a reduced GFR at baseline (defined as GFR < 60 ml/min/1.73 m^2). Correlation: -0.25 (95% CI: -0.34 to -0.15) in the RENIS cohort and -0.41 (-0.55 to -0.25) in the Pima cohort in the model adjusted for age, sex, weight and height.

References:

1. Coresh J, Selvin E, Stevens LA, et al. Prevalence of chronic kidney disease in the United States. *JAMA.* 2007;298(17):2038-2047.
2. Naghavi M, Wang H, R L, et al. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet.* 2015;385(9963):117-171.
3. Muntner P. Longitudinal measurements of renal function. *Semin.Nephrol.* 2009;29(6):650-657.
4. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc.* 1985;33(4):278-285.
5. Eriksen BO, Stefansson VTN, Jenssen TG, et al. Elevated blood pressure is not associated with accelerated glomerular filtration rate decline in the general non-diabetic middle-aged population. *Kidney Int.* 2016;90(2):404-410.
6. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int.* 1996;49(6):1774-1777.
7. Jerums G, Premaratne E, Panagiotopoulos S, Macisaac RJ. The clinical significance of hyperfiltration in diabetes. *Diabetologia.* 2010;53(10):2093-2104.
8. Tonneijck L, Muskiet MH, Smits MM, et al. Glomerular Hyperfiltration in Diabetes: Mechanisms, Clinical Significance, and Treatment. *Journal of the American Society of Nephrology : JASN.* 2017;28(4):1023-1039.
9. Melsom T, Mathisen UD, Ingebretsen OC, et al. Impaired fasting glucose is associated with renal hyperfiltration in the general population. *Diabetes care.* 2011;34(7):1546-1551.
10. Mathisen UD, Melsom T, Ingebretsen OC, et al. Estimated GFR Associates with Cardiovascular Risk Factors Independently of Measured GFR. *J.Am.Soc.Nephrol.* 2011;22(5):927-937.
11. Ruggenenti P, Remuzzi G. Time to abandon microalbuminuria? *Kidney International.* 2006;70(7):1214-1222.
12. Tu YK, Gilthorpe MS. Revisiting the relation between change and initial value: a review and evaluation. *Stat Med.* 2007;26(2):443-457.
13. Blance A, Tu YK, Gilthorpe MS. A multilevel modelling solution to mathematical coupling. *Stat Methods Med Res.* 2005;14(6):553-565.

14. Denic A, Lieske JC, Chakkera HA, et al. The Substantial Loss of Nephrons in Healthy Human Kidneys with Aging. *Journal of the American Society of Nephrology : JASN.* 2017;28(1):313-320.

15. Melsom T, Stefansson V, Schei J, et al. Association of Increasing GFR with Change in Albuminuria in the General Population. *Clinical journal of the American Society of Nephrology : CJASN.* 2016;11(12):2186-2194.

16. Denic A, Mathew J, Lerman LO, et al. Single-Nephron Glomerular Filtration Rate in Healthy Adults. *The New England journal of medicine.* 2017;376(24):2349-2357.

17. Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J.Am.Soc.Nephrol.* 2009;20(11):2305-2313.

18. Melsom T, Schei J, Stefansson VT, et al. Prediabetes and Risk of Glomerular Hyperfiltration and Albuminuria in the General Nondiabetic Population: A Prospective Cohort Study. *Am J Kidney Dis.* 2016;67(6):841-850.

19. Stefansson VTN, Schei J, Solbu MD, Jenssen TG, Melsom T, Eriksen BO. Metabolic syndrome but not obesity measures are risk factors for accelerated age-related glomerular filtration rate decline in the general population. *Kidney Int.* 2018;93(5):1183-1190.

20. Nelson RG, Bennett PH, Beck GJ, et al. Development and progression of renal disease in Pima Indians with non-insulin-dependent diabetes mellitus. Diabetic Renal Disease Study Group. *N.Engl.J.Med.* 1996;335(22):1636-1642.

21. Weil EJ, Fufaa G, Jones LI, et al. Effect of losartan on prevention and progression of early diabetic nephropathy in American Indians with type 2 diabetes. *Diabetes.* 2013;62(9):3224-3231.

22. Eriksen BO, Mathisen UD, Melsom T, et al. Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. *Kidney Int.* 2010;78(12):1305-1311.

23. Myers BD, Nelson RG, Tan M, et al. Progression of overt nephropathy in non-insulin-dependent diabetes. *Kidney Int.* 1995;47(6):1781-1789.

24. Bird NJ, Peters C, Michell AR, Peters AM. Comparison of GFR measurements assessed from single versus multiple samples. *Am.J.Kidney Dis.* 2009;54(2):278-288.

25. Fufaa GD, Weil EJ, Lemley KV, et al. Structural Predictors of Loss of Renal Function in American Indians with Type 2 Diabetes. *Clinical journal of the American Society of Nephrology : CJASN.* 2016;11(2):254-261.

26. Leffondre K, Boucquemont J, Tripepi G, Stel VS, Heinze G, Dunkler D. Analysis of risk factors associated with renal function trajectory over time: a comparison of different statistical approaches. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.* 2015;30(8):1237-1243.

27. Twisk WR. *Applied Longitudinal Data Analysis for Epidemiology.* Second ed. Cambridge, United Kingdom: Cambridge University Press; 2013.

28. Tonelli M, Klarenbach SW, Lloyd AM, et al. Higher estimated glomerular filtration rates may be associated with increased risk of adverse outcomes, especially with concomitant proteinuria. *Kidney Int.* 2011;80(12):1306-1314.

29. Turin TC, James M, Ravani P, et al. Proteinuria and rate of change in kidney function in a community-based population. *Journal of the American Society of Nephrology : JASN.* 2013;24(10):1661-1667.

30. Baba M, Shimbo T, Horio M, et al. Longitudinal Study of the Decline in Renal Function in Healthy Subjects. *PLoS One.* 2015;10(6):e0129036.

31. Thomson HJ, Ekinci EI, Radcliffe NJ, et al. Elevated baseline glomerular filtration rate (GFR) is independently associated with a more rapid decline in renal function of patients with type 1 diabetes. *J Diabetes Complications.* 2016;30(2):256-261.

32. Bjornstad P, Cherney DZ, Snell-Bergeon JK, et al. Rapid GFR decline is associated with renal hyperfiltration and impaired GFR in adults with Type 1 diabetes. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.* 2015;30(10):1706-1711.

33.   Delanaye P, Radermecker RP, Rorive M, Depas G, Krzesinski JM. Indexing glomerular filtration rate for body surface area in obese patients is misleading: concept and example. *Nephrol.Dial.Transplant.* 2005;20(10):2024-2028.

34.   Chakkera H, Denic A, Kremers W, et al. Comparison of High Glomerular Filtration Rate Thresholds for Identifying Hyperfiltration. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.* 2018.

35.   Coresh J, Turin T, Matsushita K, et al. DEcline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. *JAMA.* 2014;311(24):2518-2531.

36.   Turin TC, Coresh J, Tonelli M, et al. Change in the estimated glomerular filtration rate over time and risk of all-cause mortality. *Kidney Int.* 2013;83(4):684-691.

37.   Stefansson VT, Schei J, Jenssen TG, Melsom T, Eriksen BO. Central obesity associates with renal hyperfiltration in the non-diabetic general population: a cross-sectional study. *BMC Nephrol.* 2016;17(1):172.

38.   Holtkamp FA, de Zeeuw D, Thomas MC, et al. An acute fall in estimated glomerular filtration rate during treatment with losartan predicts a slower decrease in long-term renal function. *Kidney Int.* 2011;80(3):282-287.

39.   Wanner C, Inzucchi SE, Lachin JM, et al. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. *The New England journal of medicine.* 2016;375(4):323-334.

40.   Neugarten J, Golestaneh L. Gender and the prevalence and progression of renal disease. *Adv Chronic Kidney Dis.* 2013;20(5):390-395.

41.   Skrtic M, Lytvyn Y, Bjornstad P, et al. Influence of sex on hyperfiltration in patients with uncomplicated type 1 diabetes. *American journal of physiology. Renal physiology.* 2017;312(4):F599-f606.

42.   Krolewski AS, Skupien J, Rossing P, Warram JH. Fast renal decline to end-stage renal disease: an unrecognized feature of nephropathy in diabetes. *Kidney Int.* 2017;91(6):1300-1311.

43.   Krolewski AS. Progressive Renal Decline: The New Paradigm of Diabetic Nephropathy in Type 1 Diabetes. *Diabetes care.* 2015;38(6):954-962.

Table 1 The study population characteristics at baseline.

	The RENIS cohort N=1594			The Pima cohort N=319		
Male gender, n (%)	781	(49 %)		109	(34 %)	
Age, years	58.1	(3.8)		41.4	(10.5)	
Body mass index, kg/m^2	27.2	(4.0)		34.8	(8.2)	
Current smoker, n (%)	322	(20 %)		198.7	(82.6)	
Fasting glucose, mg/dl	95.4	(90.0 to	100.8)			
Hemoglobin A1c, %	5.5	(5.3 to	5.8)	8.5	(5.9 to	10.7)
Urinary ACR, mg/g	2.0	(0.9 to	4.8)	29.1	(10.5 to	96.1)
Systolic BP, mmHg	129.4	(17.5)		120.9	(16.0)	
Diastolic BP, mmHg	83.4	(9.8)		77.4	(9.4)	
Diabetes, n (%)	0			266	(83 %)	
Diabetes duration, years				8.5	(3.9 to	13.3)
Antihypertensive medication, n (%)	289	(18 %)		21	(7 %)	
ACE-inhibitor, n (%)	28	(1.8 %)		0		
Angiotensin 2 blocker, n (%)	132	(8.3 %)		0		
Insulin treatment, n (%)	0			59	(18.5 %)	
Oral anti-diabetic medication, n (%)	0			122	(38.2 %)	
Absolute GFR, ml/min	103.8	(19.9)		149.4	(43.2)	
GFR, ml/min/1.73m^2	93.8	(14.3)		129.1	(35.0)	

Abbreviations: RENIS, the Renal Iohexol clearance Survey; Pima; Pima Indians from Arizona, USA. BP, blood pressure; GFR, glomerular filtration rate; ACR: albumin to creatinine ratio.
Estimates are given as number (%), mean (standard deviation) or median (interquartile range).

Table 2 Association of risk factors with the GFR (Fixed effects), and the correlation between the baseline GFR and GFR decline rates (Random effects) in the Pima cohort.

	Model 1					Model 2					Model 3				
Fixed effects	Coef (ml/min)	(95	%	CI)	P value	Coef (ml/min)	(95	%	CI)	P value	Coef (ml/min)	(95	%	CI)	P value
Effects on baseline GFR[a] (intercept)															
Sex, male	13.00	(3.24	to	22.77)	0.009	-3.61	(-15.98	to	8.75)	0.6	9.11	(-2.44	to	20.66)	0.1
Age, per year	-2.22	(-2.66	to	-1.77)	<0.001	-1.90	(-2.32	to	-1.49)	<0.001	-1.88	(-2.30	to	-1.46)	<0.001
Height, per cm						1.07	(0.32	to	1.82)	<0.01	0.80	(0.12	to	1.48)	0.02
Weight, per kg						0.63	(0.44	to	0.82)	<0.001	0.66	(0.48	to	0.84)	<0.001
Diastolic BP, per mmHg											-0.15	(-0.43	to	0.12)	0.3
Systolic BP[b], per mmHg											-0.47	(-0.92	to	-0.02)	0.04
Fasting glucose, per 10 mg/dl											1.54	(1.03	to	2.05)	<0.001
Urinary ACR, per doubling											-1.01	(-2.84	to	0.83)	0.3
Diabetes duration, per year											0.27	(0.44	to	0.98)	0.5
Effects on GFR slope (per year)															
Sex	0.21	(-1.09	to	1.51)	0.8	2.09	(0.36	to	3.82)	0.02	1.30	(-0.24	to	2.84)	0.1
Age, per year	-0.02	(-0.08	to	0.04)	0.5	-0.03	(-0.09	to	0.03)	0.4	0.04	(-0.02	to	0.10)	0.2
Height, per cm						-0.16	(-0.26	to	-0.06)	<0.001	-0.11	(-0.19	to	-0.02)	0.02
Weight, per kg						0.01	(-0.01	to	0.04)	0.4	-0.01	(-0.04	to	0.01)	0.2
Diastolic BP, per mmHg											-0.073	(-0.14	to	-0.01)	0.02
Systolic BP[b], per mmHg											-0.03	(-0.07	to	0.01)	0.1
Fasting glucose, per 10 mg/dl											-0.18	(-0.24	to	-0.11)	<0.001
Urinary ACR, per doubling											-0.61	(-0.86	to	-0.35)	<0.001
Diabetes duration, per year											-0.15	(-0.24	to	-0.05)	<0.001
Random effects[c]	Estimate	(95	%	CI)		Estimate	(95	%	CI)		Estimate	(95	%	CI)	
SD of slope (GFR change), ml/min/year	3.82	(3.33	to	4.39)		3.67	(3.19	to	4.23)		2.85	(2.44	to	3.33)	
SD of intercept, ml/min	37.30	(33.90	to	41.03)		33.00	(29.86	to	36.47)		28.70	(25.86	to	31.85)	
Correlation (intercept, slope)	**-0.40**	**(-0.53**	**to**	**-0.24)**		**-0.41**	**(-0.55**	**to**	**-0.26)**		**-0.31**	**(-0.47**	**to**	**-0.13)**	
SD of the residuals	30.94	(30.15	to	31.75)		30.96	(30.17	to	31.78)		30.70	(29.92	to	31.50)	
Change in slope (ml/min/year) per 10 ml/min higher intercept	**-0.41**	**(-0.52**	**to**	**-0.26)**		**-0.46**	**(-0.59**	**to**	**-0.30)**		**-0.31**	**(-0.44**	**to**	**-0.13)**	

Abbreviations; SD: Standard deviation. BP: Blood pressure. ACR: albumin-to-creatinine ratio

[a]Absolute GFR (ml/min; not adjusted by body surface)

[b]Analysed in a separate model without diastolic BP because of collinearity

[c]Random effects reflect variation in slope and intercept between individuals that are not explained by the fixed effects or the error term.

Model 1. Sex and baseline age.

Model 2. Sex and baseline age, height and weight.

Model 3. Model 2 and baseline diastolic BP, fasting glucose, diabetes duration, urinary ACR ratio, recruitment protocol (the Diabetic Renal Disease Study (DRDS) and/or the Losartan trial), and use of antihypertensive medication, insulin or oral glucose lowering medications.

N=319 in the fully adjusted model. Smoking not included due to 140 missing values.

Conversion factor for units: Glucose in mg/dl to mmol/l; x 0.0551.

Table 3 Association of risk factors with the GFR (Fixed effects), and the correlation between the baseline GFR and GFR decline rates (Random effects) in the Renal Iohexol Clearance Survey.

Fixed effects	Model 1					Model 2					Model 3				
	Coef (ml/min)	(95	%	CI)	P value	Coef (ml/min)	(95	%	CI)	P value	Coef (ml/min)	(95	%	CI)	P value
Effects on baseline GFR[a] (intercept)															
Sex, male	22.67	(21.10	to	24.30)	<0.001	14.08	(11.99	to	16.18)	<0.001	13.55	(11.41	to	15.68)	<0.001
Age, per year	-0.75	(-0.96	to	-0.55)	<0.001	-0.82	(-1.01	to	-0.62)	<0.001	-0.80	(-0.99	to	-0.61)	<0.001
Height, per cm						0.10	(-0.03	to	0.23)	0.1	0.10	(-0.03	to	0.24)	0.1
Weight, per kg						0.50	(0.44	to	0.57)	<0.001	0.49	(0.42	to	0.56)	<0.001
Diastolic BP, per mmHg											-0.10	(-0.18	to	-0.01)	0.02
Systolic BP[b], per mmHg											-0.03	(-0.08	to	0.01)	0.2
Fasting glucose, per 10 mg/dl											2.35	(1.43	to	3.27)	<0.001
Smoking, y/n											2.92	(1.08	to	4.75)	0.002
Urinary ACR, per doubling											0.35	(-0.15	to	0.85)	0.2
Effects on GFR slope (per year)															
Sex	-0.15	(-0.38	to	0.09)	0.2	-0.19	(-0.52	to	0.14)	0.2	-0.12	(-0.47	to	0.22)	0.5
Age, per year	-0.06	(-0.09	to	-0.03)	<0.001	-0.05	(-0.08	to	-0.02)	0.001	-0.05	(-0.08	to	-0.02)	0.002
Height, per cm						0.01	(-0.01	to	0.03)	0.2	0.01	(-0.01	to	0.03)	0.4
Weight, per kg						-0.01	0.02	to	0.00)	0.2	0.00	(-0.01	to	0.01)	0.6
Diastolic BP, per mmHg											-0.004	(-0.02	to	0.01)	0.5
Systolic BP[b], per mmHg											-0.005	(-0.01	to	0.00)	0.2
Smoking, y/n											-0.31	(-0.61	to	0.00)	0.05
Fasting glucose, per 10 mg/dl											-0.09	(-0.24	to	0.06)	0.3
Urinary ACR, per doubling											-0.08	(-0.16	to	0.00)	0.05
Random effects[c]	Estimate	(95	%	CI)		Estimate	(95	%	CI)		Estimate	(95	%	CI)	
SD of slope (GFR change), ml/min/year	1.58	(1.33	to	1.87)		1.57	(1.32	to	1.86)		1.56	(1.30	to	1.87)	
SD of intercept, ml/min	14.97	(14.26	to	15.71)		14.65	(13.94	to	15.39)		13.33	(12.62	to	14.08)	
Correlation (intercept, slope)	**-0.30**	**(-0.38**	**to**	**-0.22)**		**-0.31**	**(-0.40**	**to**	**-0.23)**		**-0.31**	**(-0.40**	**to**	**-0.23)**	
SD of the residuals	5.95	(5.03	to	7.03)		5.97	(5.04	to	7.05)		5.93	(4.97	to	7.06)	
Change in slope (ml/min/year) per 10 ml/min higher intercept	**-0.32**	**(-0.36**	**to**	**-0.26)**		**-0.33**	**(-0.37**	**to**	**-0.27)**		**-0.37**	**(-0.41**	**to**	**-0.30)**	

Abbreviations; GFR: glomerular filtration; BP: blood pressure; ACR: albumin-to-creatinine ratio

[a]Absolute GFR (ml/min; not adjusted by body surface)

[b]Analyzed in a separate model without diastolic BP because of collinearity

[c]Random effects reflect variation in slope and intercept between individuals that are not explained by the fixed effects or the error term.

Model 1. Sex and baseline age.

Model 2. Sex and baseline age, height and weight

Model 3. As model 2 and diastolic BP, fasting glucose, smoking (y/n), use of antihypertensive medication and urinary ACR ratio.
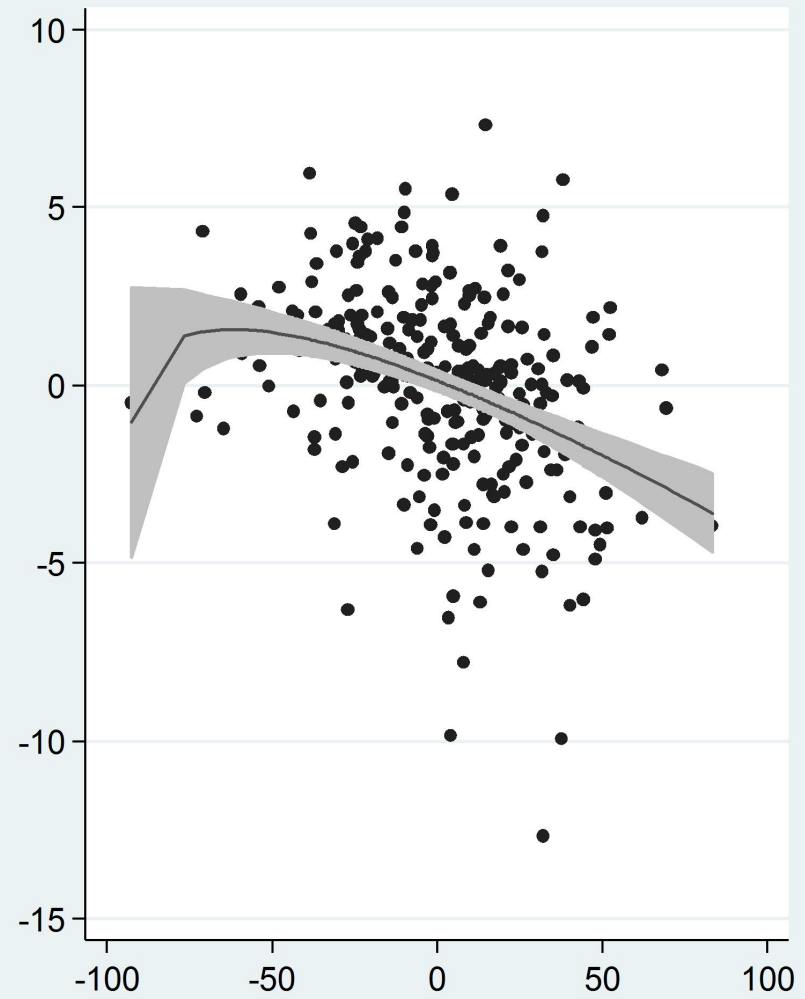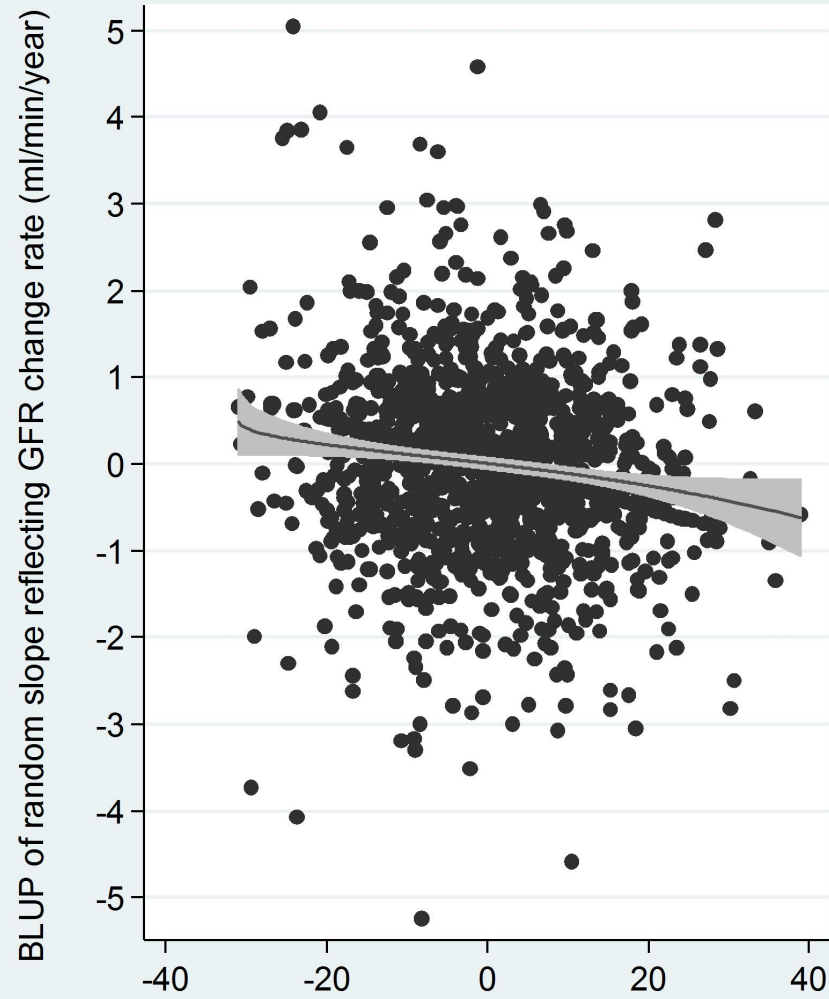
N=1586 in the fully adjusted model (Eight missing values; 3 for smoking and 5 for ACR)

Conversion factor for units: Glucose in mg/dl to mmol /l; x 0.0551.

The association between baseline GFR and GFR change rate

The RENIS cohort

The Pima cohort

BLUP of random slope reflecting GFR change rate (ml/min/year)

BLUP of random intercept; reflecting adjusted baseline GFR in ml/min (deviation from the mean)

Figure 1

Item S1: Supplementary methods and statistics.

GFR measurements

In the RENIS study, we injected five milliliters of iohexol via a Teflon catheter in the antecubital vein. The catheter was flushed with 30 ml of isotonic saline. After a calculated time period based on each person's estimated GFR, the iohexol blood sample was drawn from the same catheter.[1] GFR was calculated as described by Jacobsson.[2]

In the Pima study, diuresis was initiated by an oral water load after the bladder was emptied. A loading dose of 300 mg iothalamate plus 3 mg/kg for each 1 kg > 100 kg was given intravenously, which was followed by a continuous infusion to maintain a constant serum concentration. The bladder was again emptied after one hour, and four urine and blood samples were collected at 20-minute intervals.[3]

The serum iohexol and the urinary iothalamate concentrations were measured using high-performance liquid chromatography.[1, 3]

Statistical methods

The GFR measurements were analyzed in a two-level linear mixed regression model with a random intercept and slope using an unstructured covariance matrix for the intercept and slope.[4, 5] The linear mixed regression model divides the unexplained variance (not explained by fixed effects predictors) in the outcome variable (GFR) into different components (random effects); one related to random intercept (baseline levels) and one related to random slopes (change rates). The model further separates the variation in random effects and residual error (e.g. due to measurement error).[4, 5]

All subjects from the baseline investigations were included in the linear mixed regression analyses regardless of whether they were examined at follow-up because linear mixed models allow for missing observations at one or more time points.[4, 6] The participants had 1 to 30 measurements in the Pima cohort (median of 11 [IQR; 4-17] times) and 1 to 3 measurements in the RENIS cohort (baseline [n=1594], one follow-up [n=1299] and/or repeated follow-up [n=87]). By design, three measurements were only obtained for a random subsample in the RENIS cohort, because this suffices for estimating the three variance components in the unstructured covariance matrix of the model.

The improvement of the mixed models by including a random intercept and slope was tested by likelihood ratio (presented below).

The absolute GFR measured in ml/min at each visit was used as the dependent variable. The observation time from baseline was used as the independent time variable. The effect of an independent variable on the GFR change rate in ml/min/year (slope) was analyzed by including two-way interaction terms between the independent variable in question and the time variable. For all covariates, the models included both the main effects of each covariate and the interaction of each covariate with time. Residuals and predicted random effects were examined to ensure that they were normally distributed.

The association between the baseline GFR and subsequent GFR decline (ml/min/year) was assessed by the correlation between the random intercept and random slope.[5, 7] (This is given

as corr(obstime,_cons) in the Random-effects Parameter at the end of the statistical output using STATA). The quantitative assessment of this correlation (in ml/min/year faster decline in GFR per ml/min higher intercept (reflecting baseline GFR) was calculated by multiplying r (the correlation coefficient) with SD of the slope and dividing with the SD of the intercept.

Best linear unbiased predictors (BLUPs) of the random slopes and intercepts were calculated to display the correlation between intercept and slope.

Possible effect modification by sex was investigated by running a mixed regression model that includes sex and sex*time and allows the random effects and the correlation between them to differ by sex.[8]

Statistical tests of the random intercepts and slopes:
The total fit of the unadjusted and adjusted linear mixed model analyses for both cohorts improved by allowing for random variation in the GFR change rate (random slope) (likelihood ratio (LR); χ^2=291.51, d.f.=2, p<0.001 and LR χ^2=5.49, d.f.=2, p=0.02 for the Pima and RENIS cohorts, respectively) and by including the correlation between the GFR at baseline (random intercept) and the GFR change rate (random slope) (LR χ^2=22.5, d.f.=2, p< 0.001 and LR χ^2=14.7, d.f.=2, p<0.001 in the Pima and RENIS cohorts) for the model adjusted for age, sex, height and weight (Model 2).

Works Cited

1. Eriksen BO, Mathisen UD, Melsom T, et al. Cystatin C is not a better estimator of GFR than plasma creatinine in the general population. *Kidney Int.* 2010;78:1305-1311.
2. Jacobsson L. A method for the calculation of renal clearance based on a single plasma sample. *Clin.Physiol.* 1983;3:297-305.
3. Myers BD, Nelson RG, Tan M, et al. Progression of overt nephropathy in non-insulin-dependent diabetes. *Kidney Int.* 1995;47:1781-1789.
4. Leffondre K, Boucquemont J, Tripepi G, Stel VS, Heinze G, Dunkler D. Analysis of risk factors associated with renal function trajectory over time: a comparison of different statistical approaches. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association.* 2015;30:1237-1243.
5. Twisk WR. *Applied Longitudinal Data Analysis for Epidemiology.* Second ed. Cambrige, United Kingdom: Cambrige University Press; 2013.
6. Twisk J, de Boer M, de Vente W, Heymans M. Multiple imputation of missing values was not necessary before performing a longitudinal mixed-model analysis. *J Clin Epidemiol.* 2013;66:1022-1028.
7. Blance A, Tu YK, Gilthorpe MS. A multilevel modelling solution to mathematical coupling. *Stat Methods Med Res.* 2005;14:553-565.
8. Rabe-Hesketh SSA. Multilevel modelling of complex survey data. . *Journal of the Royal Statistical Society.* 2006;Series A:805-827.

Figure S1. Inclusion of participants in the RENIS Follow-Up Study

N = 1627

The RENIS-T6 cohort

(Baseline GFR measurement)

N = 33

Diabetes (fasting glucose ≥ 7 mmol/L and/or HbA1c ≥ 6.5%)

N = 1594 (100%)

Participants at baseline without diabetes

N = 30

Dead (N=23) or possible adverse reaction to iohexol at baseline (N=7).

N = 1564

Invited to the RENIS Follow-Up

N = 1299 (81%)

Included in the follow-up study and without diabetes (Second GFR)

N = 87

A random sample with repeated follow-up (Third GFR measurement)