Sex Differences in Age-Related Loss of Kidney Function

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- Running title: **GFR Loss in Healthy Women versus Men**
- Word count text: 3749
- Word count abstract: 246

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Keywords: Chronic kidney disease, clinical epidemiology, gender difference, glomerular filtration rate, glomerular hyperfiltration, kidney failure, microalbuminuria, renal function decline, risk factors, health status.

Abstract

Background Chronic kidney disease (CKD) is more prevalent in women, but more men receive kidney replacement therapy for kidney failure. This apparent contradiction is not well understood.

Methods We investigated sex differences in the loss of kidney function and whether any sex disparities could be explained by comorbidity or CKD risk factors. In the Renal Iohexol Clearance Survey (RENIS) in northern Europe, we recruited 1837 persons (53% women, aged 50-62 years) representative of the general population and without self-reported diabetes, CKD, or cardiovascular disease. Participants' glomerular filtration rate (GFR) was measured by plasma iohexol clearance in 2007 to 2009 (n=1627), 2013 to 2015 (n=1324) and 2018 to 2020 (n=1384). At each study visit, healthy persons were defined as having no major chronic diseases or risk factors for CKD. We used generalized additive mixed models to assess age- and sex-specific GFR decline rates.

Results Women had a lower GFR than men at baseline (mean [SD], 90.0 [14.0] mL/min per 1.73 m^2 versus 98.0 [13.7]; *P*<0.001). The mean GFR change rate was -0.96 (95% confidence interval [95% CI], -0.88 to -1.04) mL/min per 1.73m^2 per year in women and -1.20 (95% CI, -1.12 to -1.28) in men. Although the relationship between age and GFR was very close to linear in women, it was curvilinear in men, with steeper GFR slopes at older ages (nonlinear effect; *P*<0.001). Healthy persons had a slower GFR decline, but health status did not explain the sex difference in the GFR decline.

Conclusion Among middle-aged and elderly individuals in the general population, decline in the mean GFR in women was slower than in men, independent of health status.

Introduction

Chronic kidney disease (CKD) is projected to become the fifth leading cause of years of life lost in 2040.[1] In most countries, more women than men develop CKD stage G3, which is defined as a reduced glomerular filtration rate (GFR), while more men start kidney replacement therapy (KRT).[2, 3] This apparent contradiction is poorly understood, but proposed explanations include gender disparities in access to health care and KRT, biological differences between women and men leading to different GFR decline rates, bias in creatinine-based formulas to estimate the GFR and overestimation of the CKD prevalence in women.[3] In addition, sex and gender disparities in health status could cause differences in GFR loss.[3] For example, women have a lower prevalence of myocardial infarction and a longer life expectancy than men.[4] However, although cross-sectional population studies have found a higher mean GFR in healthy than in unhealthy persons,[5] it is unknown whether good health is associated with preserved GFR during aging at the individual level and whether this can explain the sex difference in CKD prevalence.[3, 5, 6]

Population-based longitudinal studies with repeated assessments of GFR in the same individuals are necessary to investigate the associations between sex, health status and age-related GFR decline. The few existing studies on GFR change rates were not population-based, did not investigate the association with health status or used equations to calculate the estimated GFR (eGFR) based on endogenous substances.[6-13] These eGFR equations are biased by non-GFR-related factors, such as muscle mass, affecting men and women

differently, particularly during aging.[14-16] Measurements of GFR by an exogenous filtration marker, e.g., iohexol, avoid these methodological problems.[17]

Accordingly, we investigated age- and sex-specific GFR decline rates in the Renal Iohexol Clearance Survey (RENIS), which is the only general population cohort with repeated measurements of GFR.[18] The aim of the study was to report a reference range for age-related GFR decline in the general population and to investigate possible sex disparities in GFR decline rates by health status.

Methods

Study sample

The Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6) was a substudy of the 6th Tromsø population-based health survey in the municipality of Tromsø in northern Norway.[19] The vast majority of the participants were Caucasian subjects as the Tromsø population has relatively few immigrants. A 40% random sample of individuals aged 50–59 years and all individuals aged 60–62 years (5464 total subjects) in Tromsø were invited to participate in the 6th Tromsø study. Of these, 3564 (65%) individuals completed the main part of the Tromsø 6, and those without self-reported diabetes, cardiovascular disease (CVD) or kidney disease were invited to participate in the RENIS-T6 (2007–2009) (Figure 1). A total of 2114 (75%) people consented to participate; 1989 were eligible for inclusion, and 1627 were included until the predetermined study size of the RENIS-T6 was obtained. The characteristics of the cohort were comparable to those of the total group of eligible recruits (n=2825) (Table S1).

Among those assessed at baseline, 1324 (83%) had follow-up GFR data in the RENIS-Follow-Up (RENIS-FU) (2013-2015), and 1174 (72%) had follow-up GFR data in the RENIS-3 (2018-2020). To counteract the tendency for internal selection bias in longitudinal cohort studies, in the RENIS-3, we also invited 353 persons who were eligible for inclusion in the RENIS-T6 but were not investigated. Because subjects were invited to participate in the RENIS-T6 in a random order until the inclusion target was met, this group (N=353) represented a random sample of all eligible persons. A total of 210 of these 353 persons were included, resulting in a total of 1384 participants with GFR measurement data in the RENIS-3 (Figure 1). The numbers of participants in the RENIS with at least 1, 2 or 3 GFR measurements were 1837, 1410 and 1088 persons, respectively (Figure S1). A random sample of 88 participants underwent two GFR measurements in the RENIS-FU to estimate day-to-day variability in the GFR. These measurements were also included in the analyses. Power calculations relevant for the current study are given in the supplementary appendix.

The research protocol was approved by the Norwegian Data Inspectorate and the Regional Ethics Committee of Northern Norway (2016/2320/REK nord). All subjects provided informed written consent.

Data collection and definition of variables.

All measurements at each visit were performed in the morning between 8-10 a.m. at the Clinical Research Unit, University Hospital of North Norway. Height and body weight were measured, and body mass index (BMI) was calculated. Blood pressure was measured 3 times, with one-minute intervals, with an automated device (A&D model UA-799). Fasting serum samples were drawn for standard laboratory measurements. Three samples of first-void morning spot urine were collected on consecutive days at each visit. The urinary

albumin and creatinine concentrations were measured in fresh urine, and the albumin to creatinine ratio (ACR) in mg/mmol was calculated for each urine specimen.[20] The median ACR value was used in the analyses.

Outcome of interest

The outcome of interest for this investigation was the decline rate of the GFR. The GFR was measured by single-sample plasma iohexol clearance at all visits, as previously described in detail.[21] In brief, five milliliters of iohexol (Omnipaque, 300 mgI/ml) was injected through a Teflon catheter placed in an antecubital vein. Serum iohexol was measured at the optimal time point for each person based on their eGFR, and the GFR was calculated with the formulas described by Jacobsson.[21] Serum iohexol was measured with high-performance liquid chromatography in the RENIS-T6 and RENIS-FU and with liquid chromatography–mass spectrometry in the RENIS-3. The measurements in the RENIS-T6 and RENIS-3 were calibrated to the RENIS-FU measurements by reanalysis of frozen samples as described in the supplemental methods. The intraindividual (day-to-day) coefficient of variation (CV) in the GFR measurement was 4.2%, as previously reported.[18]

Covariates

Data regarding comorbidities, smoking habits, medication use and hospital admissions were obtained through questionnaires at each visit. The use of lipid-lowering medications, antidiabetic drugs, cardiac glycosides (digoxin/digitoxin) or antihypertensive medications was registered as a dichotomous variable.

Tobacco smoking was categorized as current, previous or never. Hypertension was defined as systolic blood pressure (sBP) ≥140 mmHg, diastolic blood pressure (dBP) ≥90 mmHg or the use of antihypertensive medication. Diabetes was defined as self-reported diabetes, use of antidiabetic medication, or measured HbA1c ≥6.5% (≥48 mmol/mol) or fasting glucose ≥7 mmol/L.

For the 210 participants in the RENIS-3 who did not take part in the RENIS baseline investigation, we substituted baseline variables with the same variables registered in the main part of the Tromsø 6 that were collected a median of 3.8 (IQR; 2.4-4.7) months prior to the RENIS-T6. In the total study population (N=1837), there were 11 missing values for HbA1c, 1 for diabetes, 5 for smoking and 6 for albuminuria.

We measured serum creatinine and cystatin C as previously described.[21] External quality control of both assays was provided by Equalis (www.equalis.se). The eGFR was calculated from creatinine (eGFRcrea), cystatin C (eGFRcys) and both (eGFRcreacys) using the CKD-EPI equation.[22]

We defined health status as a time-dependent dichotomous variable that was ascertained concurrently with GFR measurements. A healthy person was defined as a nonsmoking person with no diabetes or hypertension, BMI <30 kg/m^2, ACR < 3.4 mg/mmol (30 mg/g), and without self-reported previous myocardial infarction, angina pectoris, coronary revascularization procedure, stroke, cancer, or use of lipid-lowering medication or cardiac glycosides.[5] Information about persons who died was obtained from the Norwegian Cause of Death Registry.

Statistical analysis

Differences in baseline characteristics between women and men were calculated using two-sample t tests or two-sample tests of proportions.

The associations between the GFR (mL/min/1.73 m^2) as the dependent variable and age, sex and time-dependent health status as independent variables were explored in linear mixed models with a random intercept and slope and an unstructured covariance matrix. Because cross-sectional age differences in the GFR (between persons) and longitudinal GFR changes (within-person change) in this cohort study converged into a common trajectory (see supplemental methods), we used chronological age as the time variable and adjusted the analyses for sex-specific baseline age.[23] A negative sign for the time coefficient indicates a decline in the GFR. The effects of sex and health status on the rate of change in the GFR were assessed by including two-way interaction terms between the variables in question and the time variable. We used generalized additive mixed models (GAMMs) to investigate a possible sex-specific nonlinear relationship between GFR and age.[24] All study participants (N=1837) were included in the linear mixed model and GAMM analyses because mixed models allow for missing observations at one or more points in time.[25, 26] The Akaike information criterion (AIC) was used to compare the fit of the different models.[27] Based on the GAMM with the best fit, the GFR change rate was calculated as the numerical time derivative of the GFR as a function of sex, age and health status. The estimated best linear unbiased predictions (BLUP) of the random slope for each person were taken to represent the interindividual distribution of the GFR change rates and used to obtain percentiles of the change rates.

We constructed smoothed histograms of the distributions of the predicted GFR change rates separately for men and women by health status. This was performed by computing kernel density estimates of the variables with the geom density-procedure in the ggplot2-package in R. Because the GFR decline curves in the best-fitting GAMM were approximately linear, and for the purpose of creating histograms, we calculated the mean predicted GFR decline rate for each person by subtracting the baseline predicted GFR from the predicted GFR at the last follow-up and dividing by the corresponding observation time for persons with at least two GFR measurements (N=1410).

Statistical analyses were performed using the mgcv and mgcViz packages in R version 4.1.0 (2021-05-18) (https://www.r-project.org/) and STATA version 16 (College Station, Texas 77845 USA).[24, 28] Statistical significance was set at $p<0.05$.

Results

The study population consisted of 1837 persons with 4423 GFR measurements. The baseline characteristics are shown in Table 1. The mean baseline age (SD) was 58 (3.8) years), and 53% of the participants were women. The mean GFR (SD) at baseline was 90.0 (14.0) mL/min/1.73 m^2 in women and 98.0 (13.7) mL/min/1.73 m^2 in men. The median follow-up time for those with GFR measurements at baseline (N=1627) was 10.7 (IQR 6.3-11.3) years. Thirty-two women (4%) and 50 men (6%) died during follow-up, ascertained on 01.01.2020. The proportions of healthy persons at the three RENIS visits were 26%, 27% and 22%, respectively, and the proportions were higher in women than in men (Table 2). Healthy women (N=242) had, on average, an 8.3 mL/min/1.73 m^2 lower GFR at baseline than healthy

men (N=179, p<0.001). A scatter plot showing the association between all GFR measurements and age in women and men separately is presented in Figure 2. One hundred and twenty-seven of 2281 GFR measurements (5.6%) in women and 48 of 2142 GFR measurements (2.2%) in men were less than 60 mL/min/1.73 m^2 (the CKD stage 3 cutoff) at any timepoint.

GFR decline

There was a statistically significant interaction between sex and GFR change rates in the linear mixed model (p <0.0001) (Model 1, Table 3). Men had a 25% steeper mean GFR decline than women (1.20 [95% CI, 1.12-1.28] vs. 0.96 [95% CI, 0.88-1.04] mL/min/1.73 m^2/year) (Model 1, Table 3). We introduced the dichotomous variable of health status into the model to see if it would modify the relationship between sex, age and GFR. Persons defined as "not healthy" had a more rapid GFR decline of 0.28 (95% CI, 0.15-0.40) mL/min/1.73 m^2/year than healthy persons (p<0.001) (Model 2, Table 3). The sex effect on the GFR change rate was very similar to that in the model without health status. There was no effect modification between sex and health status on GFR decline (p=0.34), indicating that health status had the same association with the GFR change rate in men and women.

We then included sex-specific nonlinear terms for GFR change rates in this model and found that the fit of the GAMM was improved, as indicated by a substantial decrease in the AIC relative to a model with only linear effects (from 33948 to 33929) (Model 3, Table S2). While the GFR-age relationship in women was very close to linear, the relationship in men was curvilinear, with a steeper GFR decline at older ages, as illustrated in Figure 3. The effects of sex and health status on the GFR change rate were similar in a model that added adjustments for BMI, fasting glucose, and systolic blood-pressure as continuous variables

(Model 4, Table S3). Persons defined as "unhealthy" had a higher GFR by 3.5 (95% CI, 1.7-5.2) mL/min/1.73 m^2 at 50 years of age and a steeper GFR decline by 0.24 mL/min/1.73 m^2 per year (Table S2, Table S3 and Figure 3). We also obtained similar results when we used the absolute GFR in mL/min as the dependent variable and added body weight and height as independent variables to adjust for body size (Table S4).

In analyses using the eGFRcrea and eGFRcreacys instead of the measured GFR (mGFR), there was no effect of sex on eGFR decline rates. For the eGFRcys, the effect of sex was statistically significant, although smaller than with the mGFR. For all eGFR equations, the association between health status and eGFR decline rates was weaker than that of the mGFR (Table S5).

Age-specific GFR decline rates in healthy women and men

The GFR change rates in healthy women and men as a function of age were calculated by obtaining the numerical time derivative of the GAMM in Table S2. The mean, 2.5^{th} and 97.5^{th} percentiles of the distributions of the GFR change rates are shown in Figure 4 and tabulated in Table 4. The maximum 97.5^{th} percentile was less than -0.20 mL/min/1.73 m^2 for all subjects except men between 50 and 54 years, for whom it was -0.04 mL/min/1.73 m^2 (Figure 4), demonstrating that very few persons had a stable GFR. Smoothed histograms of the distribution of individual mean predicted GFR decline rates for healthy and unhealthy women and men (N=1410 with at least two GFRs) using iohexol clearance compared to eGFR from creatinine, cystatin C, and both, are shown in Figure 5. Whereas the location and shape of the histograms for mGFR and eGFRcrea were similar, the mode of the histogram for eGFRcys was more negative and its spread greater.

Discussion

In the general population without major chronic diseases or risk factors for CKD, we found that women had a slower mean GFR decline rate than men. More women than men were defined as healthy, but this did not explain the difference in the GFR decline rate.

Previous population studies that investigated sex differences in kidney function decline, as assessed by the eGFR, yielded mixed results. Some studies reported steeper eGFR declines in men,[8-10, 29] a few reported no sex differences,[9, 13] and two studies found steeper eGFR declines in women after adjustment for CKD risk factors.[11, 30] Because these studies included patients with CVD, diabetes or CKD; used the creatinine-based eGFR; or were based on annual health check-up data, the results may not reflect GFR decline rates in the healthy general population. We obtained different results using the eGFR from creatinine or cystatin C compared to the mGFR, suggesting that non-GFR-related factors influence eGFR decline rates in men and women differently.

CKD stage 3 (GFR of 30-60 mL/min/1.73 m^2) accounts for a large proportion of the total CKD population, with a significantly higher prevalence in women than in men, ranging from 10-100% higher in women in different countries.[3] Overdiagnosis of CKD stage 3a (GFR of 45-60 mL/min/1.73 m^2) by the eGFR has been suggested as an explanation.[3, 31, 32] We found that healthy, middle-aged women had an 8.3 mL/min/1.73 m^2 lower GFR than healthy men at baseline, indicating true sex differences in kidney function. As a consequence, more healthy women in these age groups had CKD stage 3a (GFR of 45-60 mL/min/1.73 m^2) (Figure 2). However, as women had a slower GFR decline, women maintained a higher mean GFR than men at ages older than 72 years (Figure 3). If the steeper GFR decline in men continues at lower GFR levels in both healthy and diseased individuals, as suggested by others,[29, 33, 34] it

may contribute to the higher prevalence rates of stage 5 CKD (GFR<15 mL/min/1.73 m^2), dialysis and kidney transplantation in men.[3] It may also contribute to lower life expectancy in men than in women because rapid GFR loss (> 3 ml/min/1.73 m^2/year) and GFR < 45 mL/min/1.73 m^2 are both independent risk factors for all-cause mortality in older people from the general population.[35, 36]

The sex differences described above and the GFR loss observed even in healthy persons have relevance for the ongoing discussion about whether the CKD definition should be age- and sex-adjusted.[31, 37] If normality is based on the distribution of GFR values in healthy persons, our findings support age- and sex-specific cutoff values for defining CKD. For example, a 70-year-old healthy woman with a GFR of 59 mL/min/1.73 m^2 and no albuminuria is currently labeled with CKD stage 3a, even though her GFR is within the 95% age-and sex-specific reference range in European populations.[5, 31] A CKD diagnosis may cause anxiety and referral to a specialist health care center, but according to our study her risk of accelerated GFR loss is low, and the risk of end stage kidney disease has been found to be minimal.[37, 38] Conversely, a GFR of 65 mL/min/1.73 m^2 in a man younger than 50 years does not fulfill the CKD criteria, although his GFR is clearly abnormal,[5] and his life-time risk of progression to CKD stage 4 and 5 may be significant. However, the association between GFR levels and the risk of morbidity and mortality should also be considered.[37] For people older than 65 years, the relative risk is small, if any, until the GFR has fallen below 45 mL/min/1.73 m^2.[35, 37, 39] Studies on measured GFR that include sex-specific morbidity or mortality endpoints are needed to decide whether the CKD definition should be adjusted for age and sex.

Differences in nitric oxide metabolism and oxidative stress between women and men, as well as the influence of sex hormones, have been proposed as explanations for the sex

difference in the GFR.[3, 40] The female participants in our study were 50-62 years old at baseline, and the sex difference in GFR decline rates was more prominent at older ages, making the influence of female sex hormones unlikely. Although the majority of experimental studies support deleterious renal effects of testosterone, a delayed progression of CKD in hypogonadal men treated with low-dose testosterone has been reported.[41, 42] Whether a gradual loss of testosterone in men during aging may influence the GFR decline rate and the risk of CKD is unknown.[40-45]

We found that persons classified as unhealthy had a higher GFR at a younger age and a steeper GFR decline. A possible explanation may be the increased prevalence of an abnormally elevated GFR, i.e., hyperfiltration, associated with some of the conditions included in our definition of "unhealthy". Hyperfiltration leads to podocyte stress, glomerulosclerosis and progression of CKD. In the general population, it is associated with diabetes, obesity, prediabetes, hypertension, and subsequent GFR loss and may also be a risk factor for cardiovascular events and all-cause mortality.[46-51] However, adjustment for health status, BMI, blood pressure, and fasting glucose did not influence the effect modification by sex on GFR decline rates, which makes hyperfiltration a less likely explanation for sex differences.

Previous longitudinal studies indicated that the GFR is preserved or increases with age in a significant proportion of healthy persons.[6-13, 52] These studies used the eGFR or creatinine clearance rate; some were limited by short follow-up times, and some calculated the GFR change rate as the difference between GFR measurements divided by time. The variation in the GFR change rates calculated by this method includes both interindividual variation and random measurement error, resulting in a wide distribution and a higher proportion of

change rates greater than zero, i.e., a preserved or increased GFR. The generalized additive linear mixed model used in this study estimated interindividual variation and random error separately and found very few persons with a preserved GFR during aging (Figure 4 and 5).

Although we did not observe sex-differences in eGFR decline rates using creatinine, we found that the mean and distribution of eGFRcrea decline rates were more comparable to the mean and distribution of mGFR than eGFRcys (Figure 5). While the annual mean decline rates were approximately -1.0 mL/min/1.73 m2 for women and men using eGFRcrea, for eGFRcys they were -2.2 and -2.3 mL/min/1.73m^2 for women and men, respectively. Since the quality of the cystatin C assay has been under continuous external quality control, influence from non-GFR related factors seems the most likely explanation for the discrepancy.

The strength of this study was the repeated GFR measurements in a well-described cohort representative of the general population without pre-existing diabetes or CVD. The participation rate was fair, and the day-to-day variation in GFR measurements was low.

There are also some limitations. Study participants were of European ancestry, limiting the generalizability. The average GFR levels for men and women in this study were higher than those reported in some other general population studies. However, we aimed to study age-related GFR decline in healthy people, and GFR levels were comparable to studies in healthy kidney donors.[53, 54] Although relatively few participants were lost to follow-up, we cannot exclude bias due to a higher drop-out rate among those with poor health or the retention of healthy persons. Bias due to competing risk of death was unlikely because only 5% of participants died during follow-up. We chose a stringent definition of “healthy”, but we cannot exclude that some mechanisms contributing to GFR decline in this category should be classified as pathological rather than age-related. For example, the long-term effect of

glucose and blood pressure levels within the normal range on GFR variations is poorly defined. Any such pathological mechanism would need to have sex-specific effects in healthy persons to change our conclusion of differences in GFR decline rates between the sexes. Because we did not find any interaction between sex and health status on GFR decline, this possibility seems less likely.

In conclusion, we found that men had a steeper GFR decline rate than women in a representative sample of the general population aged between 50-75 years and without diabetes, CKD or CVD at baseline. Good health did not explain the sex difference in the decline rate.

Authors` Contributions

Toralf Melsom: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft

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Acknowledgements

We thank the staff at the Clinical Research Unit, University Hospital of North Norway, for their assistance in planning the study, performing the procedures, and collecting the data according to the GCP standard. We thank all participants for their participation in the study. The RENIS was funded by the University Hospital of North Norway and Northern Norway Regional Health Authority. The RENIS-FU was also supported by an unrestricted grant from Boehringer-Ingelheim, not related to this work.

Disclosures

T. Melsom reports Honoraria: Novo Nordisk Norway AS, and lecture at a local meeting. V. Stefansson reports Ownership Interest: Biosergen, Telenor, Volvo, Norwegian, NordNet, Storebrand, Norsk Hydro, Komplett; Research Funding: Boehringer-Ingelheim gave a grant to the RENIS-FU study.; and Other Interests or Relationships: The Norwegian Medical Association (NMA), UiT, and The Arctic University of Norway. T. Jenssen reports Consultancy: NovoNordisk, Boehringer Ingelheim, AstraZeneca, Bayer, EliLilly; Honoraria: AstraZeneca, Boehringer Ingelheim, NovoNordisk, Bayer; and Advisory or Leadership Role: Medical Advisor, Norwegian Association of Diabetes. M. Solbu reports Honoraria: AstraZeneca; and

Other Interests or Relationships: Norwegian Society of Nephrology, ERA-EDTA, ISN, and Norwegian Society of Hypertension.

Funding/Support

The RENIS studies were funded by the Northern Norway Regional Health Authority and the University Hospital of North Norway (Grant / Award Number: 'HNF1422-18','SFP 1100- 13'). The funding source had no role in the design and conduct of the study.

Data Sharing Statement

The data underlying this article cannot be shared publicly because this was not included in the research permission due to ethical considerations and the privacy of individuals who participated in the study. The data can be shared on request as part of a research collaboration. Please contact the corresponding author, Toralf Melsom (toralf.melsom@unn.no), or the last author, Bjørn Odvar Eriksen (bjorn.odvar.eriksen@unn.no).

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Supplemental Table of Contents

Supplemental Figure 1. The total study population with at least one GFR measurement in the Renal Iohexol Clearance Survey (RENIS)

Table 1. Baseline characteristics of the RENIS cohort

Baseline characteristics	All (n=1837)	Women (n=974)	Men (n=863)
Age (SD), years	58.2 (3.8)	58.1 (3.9)	58.3 (3.8)
Body mass index (SD), kg/m2	27.2 (4.0)	26.7 (4.4)	27.8 (3.4)
Obese (BMI>30), n (%)	401 (22 %)	187 (19 %)	214 (25 %)
Systolic BP (SD), mmHg	129.9 (17.9)	125.8 (17.6)	134.4 (17.1)
Diastolic BP (SD), mmHg	82.7 (10.2)	79.7 (9.8)	86.0 (9.5)
Use of antihypertensive medication, n (%)	361 (20 %)	176 (18 %)	185 (21 %)
Hypertension, n (%)	762 (41 %)	327 (34 %)	435 (50 %)
Hemoglobin A1c (SD), mmol/mol	37.2 4.0	37.1 3.9	37.4 4.0
Diabetes[a], n (%)	38 (2 %)	16 (2 %)	21 (2 %)
Current smoking, n (%)	384 (21 %)	216 (22 %)	168 (20 %)
Total cholesterol (SD), mmol/L	5.68 (0.95)	5.75 (0.96)	5.60 (0.94)
Lipid lowering medication, n (%)	121 (7 %)	73 (7 %)	48 (6 %)
Albuminuria (ACR>3.4 mg/mmol)[b], n (%)	24 (1 %)	11 (1 %)	13 (1.5 %)
Measured GFR[b] (SD), mL/min/1.73 m^2	93.9 (14.4)	90.0 (14.0)	98.0 (13.7)

Abbreviations: RENIS, Renal Iohexol-clearance Survey; GFR, glomerular filtration rate; SD, standard deviation; BP, blood pressure; ACR, albumin-to-creatinine ratio. Estimates are given as the mean (SD) or number (percent).
[a]Numbers are undiagnosed diabetes based on fasting glucose or HbA1c. Those with self-reported diabetes were excluded at baseline. [b]ACR> 30 mg/g. [c]GFR measured using single-sample iohexol clearance. GFR was not measured at baseline for the additional 210 persons included in RENIS-3 who did not attend RENIS-T6.
There were 11 missing values HbA1C, 1 for diabetes, 5 for current smoking and 6 for albuminuria.

Table 2. Proportion of healthy women and men at baseline and follow-up

	RENIS-T6 (2007-2009)				**RENIS-Follow-Up (2013-2015)**				**RENIS-3 (2018-2020)**			
	All (n=1627)	Women (n=826)	Men (n=801)	p[b]	All (n=1324)	Women (n=667)	Men (n=657)	p[b]	All (n=1384)	Women (n=744)	Men (n=640)	p[b]
Healthy[a], n	421 (26%)	242 (30%)	179 (22%)	0.001	360 (27%)	197 (30%)	163 (25%)	0.05	299 (22%)	176 (24%)	123 (19%)	<0.05

[a]Healthy, defined as a non-smoking person without diabetes, hypertension, myocardial infarction, angina pectoris, coronary revascularization procedures, stroke, cancer and use of lipid-lowering medication or digoxin, as well as a body mass index <30 kg/m^2 and urinary albumin-to-creatinine ratio < 3.4 mg/mmol (30 mg/g). [b]Chi2-test for difference between women and men.

Table 3. Associations between sex, health status and GFR change rates in linear mixed models.

	Model 1			Model 2		
	mL/min/1.73 m^2 per year	(95 % CI)	P value	mL/min/1.73 m^2 per year	(95 % CI)	P value
Women	-0.96	(-0.88 to -1.04)	<0.001	-1.04	(-1.12 to -0.95)	<0.001
Men	-1.20	(-1.12 to -1.28)	<0.001[a]	-1.26	(-1.18 to -1.35)	<0.001
Healthy[b]				0.28	(0.15 to 0.40)	<0.001
Difference between men and women	-0.24	(-0.12 to -0.35)	<0.001	-0.23	(-0.11 to -0.34)	<0.001

Both models were adjusted for age at baseline, with separate terms for women and men.

[a]P <6e-5 for effect modification by sex.

[b] Healthy, defined at each visit as no cardiovascular disease, cancer, diabetes, hypertension, smoking, lipid-lowering medication or digoxin, as well as a body mass index <30 kg/m^2 and urinary albumin-to-creatinine ratio < 3.4 mg/mmol (30 mg/g).

Table 4. Age-specific annual GFR change rates for healthy women and men

Age-group	Women			Men		
		Percentiles			Percentiles	
	Mean	2.5th	97.5th	Mean	2.5th	97.5th
50-54	-0.72	-1.29	-0.20	-0.58	-1.15	-0.06
55-59	-0.72	-1.30	-0.20	-0.73	-1.30	-0.21
60-64	-0.79	-1.36	-0.27	-0.98	-1.55	-0.46
65-69	-0.86	-1.43	-0.34	-1.22	-1.79	-0.70
70-75	-0.88	-1.46	-0.37	-1.52	-2.09	-1.00

The values are means (mL/min/1.73 m^2/year) for each five-year interval. The 95% reference intervals were estimated from the best linear unbiased predictions of the random slopes of the generalized additive model in Table S2.

Figure Legends

Figure 1. Inclusion of participants in the Renal Iohexol Clearance Survey (RENIS)

Figure 2. The association between GFR and age for women and men. Blue dots are healthy subjects and red dots are subjects defined as not healthy (prevalent comorbidity or CKD risk factors). Age was used as the time variable (baseline age + follow-up time).

Figure 3. Mean GFR decline with age for women and men by health status ("healthy" in green and "not healthy" in gray). The lower panel depicts the mean GFR decline with age for healthy women (light green) vs. healthy men (dark green). Calculated using a generalized additive mixed model (Model 3, Table S2).

Figure 4. Sex-specific GFR change rates for healthy women and men as a function of age with 95% reference intervals.
The solid lines represent the GFR change rates for women (red) and men (blue).
The dashed lines represent the 95% reference intervals estimated from the best linear unbiased predictions of the random slopes of the generalized additive mixed model in Table S2.

Figure 5. Smoothed histograms of the distribution of individual predicted mean GFR decline rates. GFR change rates were measured using iohexol clearance (panel A) and estimated from creatinine (panel B), cystatin C (panel C), and creatinine and cystatin C (panel D) for healthy (solid) and unhealthy (dashed) women (red) and men (blue), for participants with at least one follow-up (N=1410). Predicted by the generalized additive mixed model in Model 3, Table S2 for measured GFR, and Table S5 for eGFR.

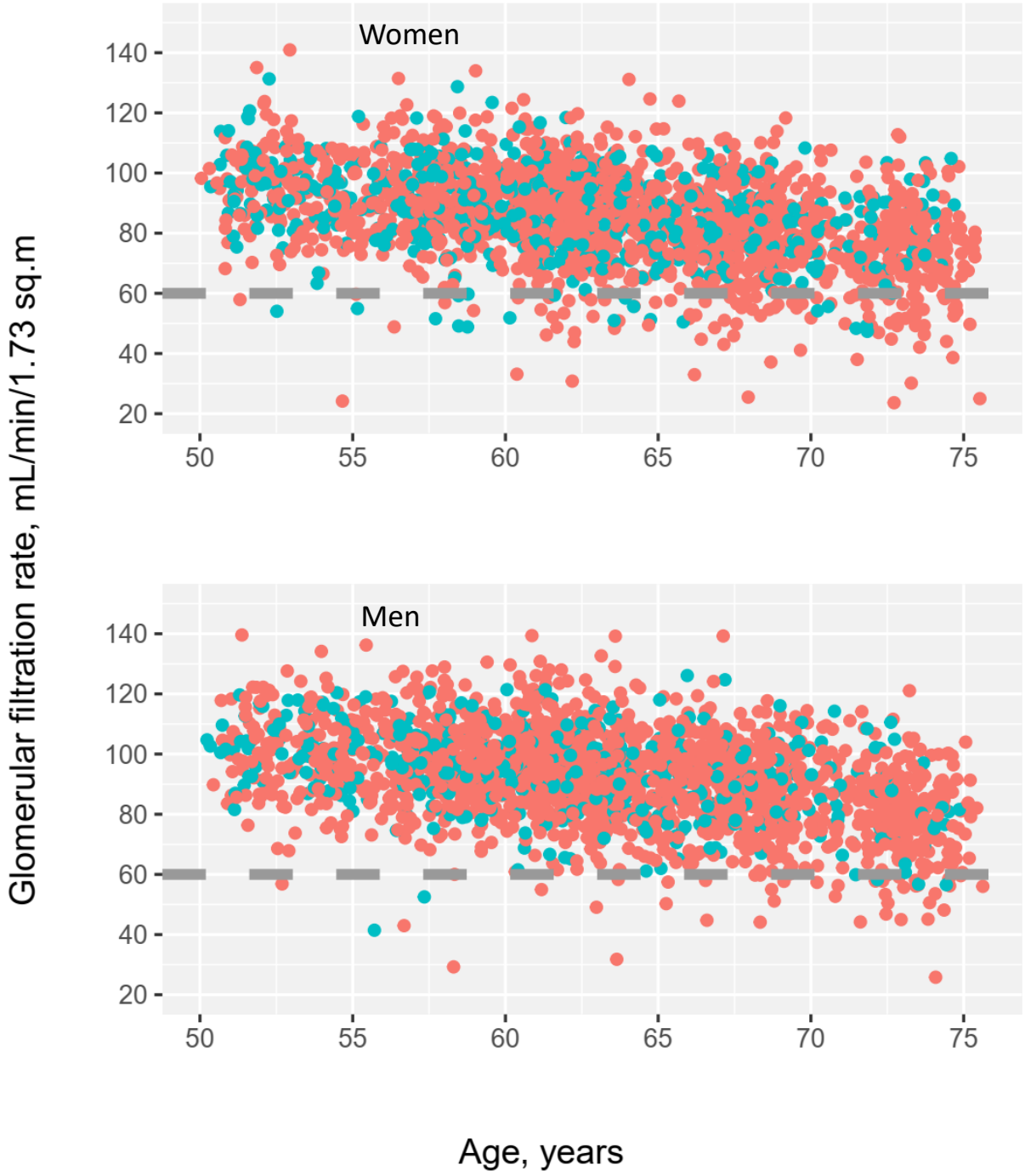
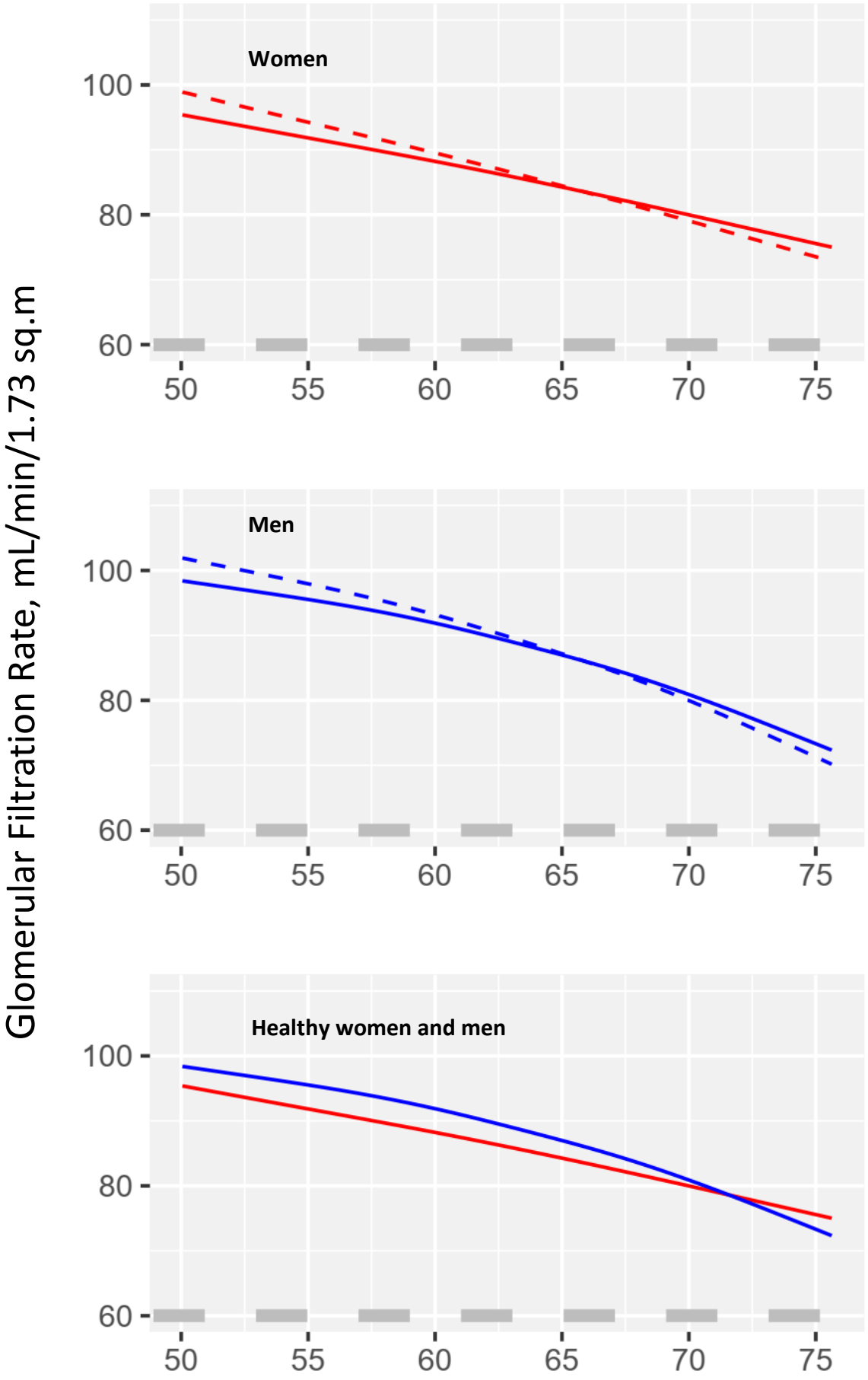


Figure 2. The association between GFR and age for women and men. Blue dots are healthy subjects and red dots are subjects defined as not healthy (prevalent comorbidity or CKD risk factors). Age was used as the time variable (baseline age + follow-up time).


Women
Men
Healthy women and men
100
80
60
50
55
60
65
70
75
Glomerular Filtration Rate, mL/min/1.73 sq.m
Age, years

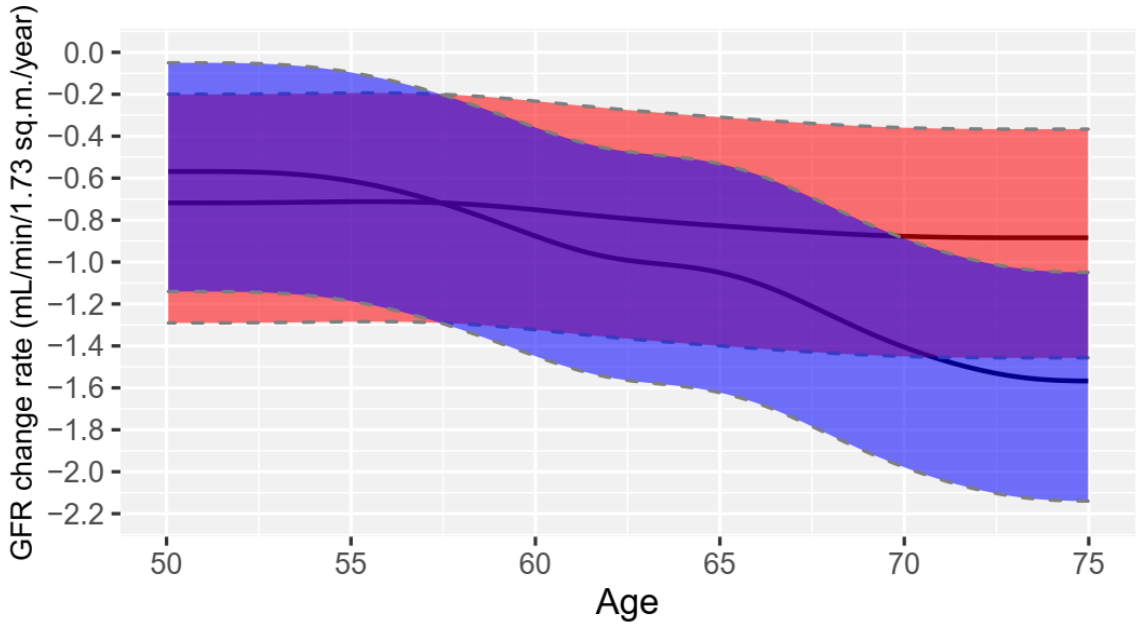


Figure 4. Sex-specific GFR change rates for healthy women and men as a function of age with 95% reference intervals.

The solid lines represent the GFR change rates for women (red) and men (blue).

The dashed lines represent the 95% reference intervals estimated from the best linear unbiased predictions of the random slopes of the generalized additive mixed model in Table S2.

Alternative colors:

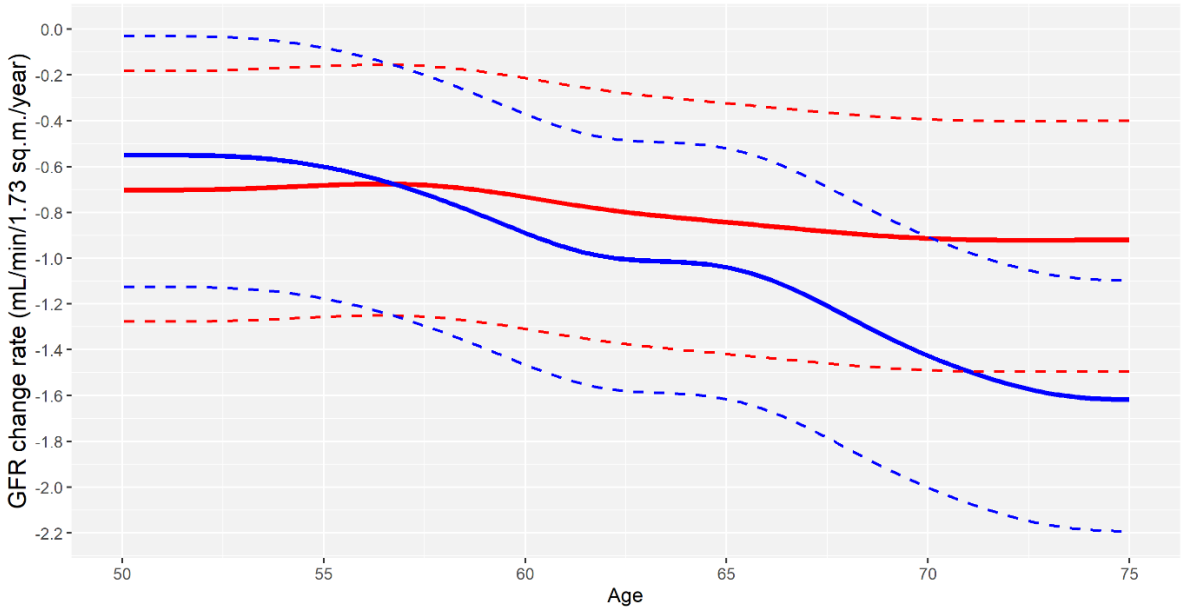


Figure 4. Sex-specific GFR change rates for healthy women and men as a function of age with 95% reference intervals.

The solid lines represent the GFR change rates for women (red) and men (blue).

The dashed lines represent the 95% reference intervals estimated from the best linear unbiased predictions of the random slopes of the generalized additive mixed model in Table S2.

Supplementary Appendix

Supplement to Melsom T et al. Sex differences in age-related loss of kidney function.

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Supplementary Methods

Using age as the time variable - Convergence

This investigation of longitudinal data from RENIS aims to estimate GFR change rates. Because the cohort included persons at different ages at baseline, the observed GFR changes will reflect both cross-sectional age differences and longitudinal age changes, i.e. changes in GFR both within and between persons. The models used in the investigation assume that the cross-sectional age differences and longitudinal age changes converge onto a common trajectory.

Sliwinski et al provide a method for checking if this assumption is met.[1] The method estimates the parameter ω, which is the mixing weight that controls the relative contribution of the cross-sectional and longitudinal age slope (to the estimation of the age slope). The higher the value of ω, the more the age slope would reflect the cross-sectional information about between-person age differences, and the lower the value of ω the more the age slope would reflect the longitudinal information about age changes. A cross-sectional study corresponds to $\omega = 1$ and an age-homogenous longitudinal study to $\omega = 0$. $\omega < 0.20$ indicates that the estimated age slope primarily reflects longitudinal information.

With Sliwinski et al's equations,[1] we estimated the convergence age slope in the RENIS cohort at -1.06 mL/min/1.73 m^2/year (95% CI -1.11 to -1.00 mL/min/1.73 m^2/year), the between-person age difference at -0.95 mL/min/1.73 m^2/year (95% CI -1.07 to -0.83 mL/min/1.73 m^2/year) and the within-person change rate at -1.08 mL/min/1.73 m^2/year (95% CI -1.14 -1.02 mL/min/1.73 m^2/year). The difference between the two last estimates was 0.13 mL/min/1.73 m^2/year (95% CI -0.00 to 0.26 mL/min/1.73 m^2/year), i.e. not statistically different from zero.

Based on these estimates, we calculated ω in the present study at 0.18, which means that the GFR change rates in models with chronological age as the time variable primarily reflects the within-person GFR change rate. We included chronological age at baseline as the independent variable in all models to adjust for the between-person age differences in GFR and make the estimated GFR change rates reflect within-person changes, as recommended by Sliwinski et al.[1]

Power calculations

Software for power calculations of GAMMs is not readily available. Accordingly, a power calculation for the hypothesis that sex has a statistically significant effect on the GFR decline rate was explored by simulation of 2000 iterations in a linear mixed model without non-linear functions. The parameters for the simulation were taken from the results of a linear mixed model of RENIS baseline and follow-up data. Variance parameters for a model including the most important predictors of GFR decline rate were used to assess the possibility of a new dichotomous predictor to detect an effect on the remaining inter-individual variation of the GFR decline rate with an expected sample size of 1550 persons. The power of detecting an effect of -0.13 mL/min/year or lower of the predictor with negligible correlation with the other independent variables was calculated. It was found to be 0.88, assuming α=0.05. Compared to the mean GFR change rate, the power of the study to detect clinically significant effects was judged sufficient. Since the final number of included persons (N=1384) was lower than expected, the actual power was somewhat lower than in this simulation.

Calibration of the HPLC and LC-MS/MS analyses of serum iohexol

In RENIS-T6 and RENIS-FU, serum iohexol was analyzed by HPLC as described in previous publications.[2,3] In 2017, the Department of Medical Biochemistry at the University Hospital of North Norway replaced HPLC with LC-MS/MS as its standard assay for analyzing serum iohexol, which was subsequently used in RENIS-3 (the method is described below). This made it necessary to establish a calibration equation for the conversion of results between the two methods. Serum samples from the single sample iohexol clearance studies of all the 1324 participants in the Renal Iohexol Clearance Survey Follow-Up (RENIS-FU) in 2013 – 2015 had been stored at -80 °C. A random sample of this material was thawed and reanalyzed with LC/MS concurrently with samples from RENIS-3.

Calculation of sample size for calibration

To calculate the necessary sample size to calibrate between HPLC and LC/MS with Deming regression, we followed the method of Linnet.[4] Although this method was published before Martin developed his as iteratively reweighted general Deming regression, we assume that it is valid for this form of Deming regression as well.[5]

We assumed that the standard deviation (SD) for both the new and old iohexol-analyses were proportional to the values, i.e. that the coefficient of variation (CV) was constant. In our laboratory, the analytical CV during the study periods was 3% in RENIS-T6 and 3.1% in RENIS-FU.[3] The range of the iohexol values in both studies combined was 24 to 165 mg/L. However, the distribution was skewed, and 98% of the observations were located in the interval of 32 to 96 mg/L.

Basing our calibration on a sample from this interval, we obtained a range ratio (maximum divided by minimum observed original iohexol measurement) of 3. A higher range ratio requires a lower sample size to detect a deviation with the same power. We wanted to detect an intercept less than 2.0 and an absolute slope difference of greater than 0.025 (arbitrarily chosen low values). The midpoint of the interval of interest was 64 mg/L. This gives a delta-intercept of (2.0/(0.03 x 64))=1.04; and a delta-slope of (0.025/0.03)= 0.83. Interpolating in Linnet's Table 2 with these parameters gave a necessary sample size of roughly 200. Because the distribution was normal and not uniform, this was multiplied by a factor of 1.3 to 1.5 to obtain the correct sample size for the slope, giving a total of 260 to 300. Because a precise estimate of the parameters was essential, we chose a sample size of 300. These were sampled randomly among all RENIS-FU-samples with iohexol-values in the interval 32 to 96.

Calibration equation

Sufficient serum for analysis with LC/MS was found for 287 of the 300 randomly selected participants. A scatterplot of HPLC vs. LC/MS results identified four extreme outliers which were excluded from the analysis.

Iohexol measured in RENIS-FU with HPLC was used as the dependent variable and iohexol measured in RENIS-3 was used as the independent variable in Deming regression in STATA 15. Log-transformation of the variables was found to give the best fit. The ratio of measurement error variances for the two variables was set at 1. The result of the Deming regression was:

Deming regression — Number of obs = 283

	Mean	Std. Dev.
liohexol_f~1	3.9208	.19928
liohe~202004	3.8201	.19599

Variance ratio = 1
Root MSE = .0327593

	Coefficient	Jackknife std. err.	t	P>|t|	[95% conf.	interval]
liohexol202004	1.017258	.015119	67.28	0.000	.9874976	1.047018
_cons	.0348231	.0582042	0.60	0.550	-.0797468	.149393

Accordingly, the calibration equation was:

$$iohexol_{RENIS-FU} = e^{0.035} \times iohexol_{RENIS-3}{}^{1.017}$$

This equation was used to calibrate serum iohexol measured in RENIS-3 to RENIS-FU. We have previously performed a similar calibration of iohexol measured at baseline in RENIS-T6 to RENIS-FU.[3] This makes iohexol measured across all three rounds of RENIS comparable.

LC-MS/MS measurement of iohexol

Chemicals and solutions

Iohexol, Iohexol-d5, and iohexol for quality controls (QCs) were obtained from Toronto Research Chemicals Inc. (Ontario, Canada) and TCI Chemicals (Tokyo, Japan). LC-MS grade methanol was purchased from Honeywell™ Riedel-de Häen™ (Seelze, Germany) and LC-MS grade formic acid from Fluka (Sigma-Aldrich, St. Louis, MO). Ultrapure water (18.2 MΩ) was obtained from a Millipore Advantage Milli-Q system (Millipore SAS, Molsheim, France).

Determination of iohexol in human serum

We prepared two stock solutions of iohexol in methanol and stored them at -30 °C. A 6-point calibration curve and two QCs for iohexol were constructed in drug-free serum (1-240 mg/l) and a Tecan Freedom Evo 200 (Männedorf, Switzerland) liquid handling workstation was used for sample preparation. We prepared the calibrators, QCs, and samples (50 µL) by adding 50 µl internal standard (aqueous iohexol-d5, 3.3mg/L) in a 96-well MegaBlock® 1.2 mL, PP, (Sarstedt, Germany). 0.5 mL of ice-cold methanol was added to each of the wells. The plate was mixed on a Bioshake (Quantifoil Instruments, Jena, Germany) at 1500 rpm for 3 min and centrifuged at 240 x g for 8 min (Hettich Rotina 320R, Tuttlingen, Germany). Then, 100 µl of the supernatant was transferred to a 96-well collection plate (Waters, Milford, MA). After sealing of the plate, 0.1 µl of the supernatant was analyzed by LC-MS/MS using a Waters Acquity UPLC I-Class FTN system with an autosampler and a binary solvent delivery system (Waters, Milford, MA) interfaced to Waters Xevo TQ-S benchtop tandem quadrupole mass spectrometer (Waters, Manchester, UK). The chromatography was performed on a 2.1 x 100 mm Waters Acquity Cortecs® T3, 1.6 µm column. Eluent A consisted of 0.1% formic acid in water and eluent B of 0.1% formic acid in methanol. Gradient elution was performed with 2% B at the start and had a linear increase to 60% B until 0.6 min, a linear increase to 98% B until 1.5 min, and re-equilibration until 2.7 min with 1% B. The flow rate was 0.3 mL/min and the column temperature was maintained at 50 °C. The mass spectrometer was run in positive electrospray ion mode and the spray voltage was set to 0.9 kV. The system was controlled by MassLynx version 4.1 software. The desolvation gas temperature was 500 °C, the source temperature was 150 °C, desolvation gas flow was 1000 L/h, the cone gas flow was 150 L/h, and the collision gas pressure was 4 x 10-3 mBar. For quantitative analysis of iohexol we used the following multiple reaction monitoring (MRM) transitions (bold

transitions are qualifiers): m/z 821.9->803.8/**602.4** and 826.9->808.8/**607.5** (iohexol and iohexol-d5).

Precision and accuracy

The method was validated and found to be linear from 1.5 to at least 240 mg/L (r2 > 0.999). The lower limit of quantification was 0.5 mg/L (0.1 µl injection volume). The coefficient of variation (CV) for intraday precision was 2.8 % calculated by assaying three samples (low, medium, and high concentration) six times on the same day. Accuracy for recovery test was 91.1-107.9 % (9 levels, n = 3 for each). Between-day CV for iohexol was 5.4% on four consecutive days. The quality is assured through the Equalis external quality assessment program for iohexol four times a year.

Table S1. Characteristics of all persons invited to the Renal Iohexol Clearance Survey (RENIS) and of persons actually included in each of its three waves as registered in the main part of the sixth Tromsø Study (before RENIS baseline).

	RENIS-T6			RENIS-FU			RENIS-3			All invited persons	
			P-value			P-value			P-value		
N	1627	(100)		1324	(100)		1384	(100)		2825	(100)
Age (SD), years	57.8	(3.8)	<0.001	57.7	(3.9)	<0.001	57.9	(3.9)	0.08	58.0	(3.9)
Sex, men	801	(49)	<0.001	657	(50)	<0.001	640	(46)	0.4	1283	(45)
Body mass index (SD), kg/m2	26.9	(4.0)	0,002	26.9	(3.9)	0.2	26.8	(3.9)	0.3	26.7	(4.1)
Current smoking, n (%)	345	(21)	0.6	255	(19)	0.01	265	(19)	0.002	609	(22)
Systolic BP (SD), mmHg	134.5	(20.4)	0.1	134.1	(20.0)	0.7	133.6	(20.0)	0.4	134.0	(20.1)
Diastolic BP (SD), mmHg	79.7	(10.6)	<0.001	79.6	(10.6)	0.006	79.1	(10.7)	0.9	79.1	(10.6)
Use of antihypertensive medication, n (%)	261	(16)	0.08	206	(16)	0.4	208	(15)	0.9	424	(15)
LDL cholesterol (SD), mmol/L	3.8	(0.9)	0.03	3.7	(0.9)	0.02	3.8	(0.9)	0.1	3.8	(0.9)
HDL cholesterol (SD), mmol/L	1.5	(0.4)	0.01	1.6	(0.4)	0.09	1.6	(0.4)	0.5	1.6	(0.4)
Lipid-lowering medication, n (%)	110	(7)	0.2	90	(7)	0.3	90	(7)	0.6	177	(6)
Hemoglobin A1c (SD), mmol/mol	5.6	(0.4)	0.4	5.5	(0.4)	0.02	5.5	(0.4)	0.01	5.6	(0.4)
GFRcrea[a] (SD), mL/min/1.73m2	94.4	(9.8)	0.002	94.3	(9.7)	0.05	93.8	(9.9)	0.6	93.9	(9.9)
GFRcys[a] (SD), mL/min/1.73m2	101.4	(12.6)	0.002	101.6	(12.5)	0.001	101.3	(12.6)	0.02	100.8	(12.6)
GFRcreacys[a] (SD), mL/min/1.73m2	100.0	(11.2)	<0.001	100.0	(11.0)	0.003	99.6	(11.2)	0.2	99.3	(11.2)
Albuminuria (ACR>3.4 mg/mmol)[b], n (%)	24	(1.5)	0.8	19	(1.4)	0.7	13	(0.9)	0.01	43	(1.5)

Values are given as n (%) or mean (standard deviation). P-values are given for tests of difference between included and not included persons in each wave of RENIS. Tests were performed with ANOVA or chi square-test for continuous and dichotomous variables as appropriate. Variables presented in this table were registered in the main part of the sixth Tromsø Study, conducted 5.2 (IQR; 3.0-6.2) months before RENIS-T6.

[a]GFR is estimated based on the CKD-EPI (2012) Equations from creatinine, cystatin C, or both.

[b]ACR> 30 mg/g

Table S2. The relationship between age, sex, health status, and GFR in the generalized additive mixed model.

	Model 3		
	β	(95 % CI)	P value
Linear effects on baseline GFR.			
Intercept, mL/min/1.73 m2	86.0	(84.1 to 87.9)	
Male sex	2.46	(-0.36 to 5.28)	0.09
Being healthy[a]	-3.47	(-5.24 to -1.70)	<0.001
Linear effects on GFR change rate, mL/min/1.73 m^2/year			
Being healthy[a]	0.22	(0.10 to 0.35)	<0.001
Nonlinear effects	Effective degrees of freedom[b]		
Age (time variable), y	2.09		<0.001
Interaction between age and male sex	2.82		<0.001
Aikaike Information Criterion[c]	33929		

All models were adjusted for sex-specific baseline age.

[a]Healthy is defined as having no cardiovascular disease, cancer, diabetes, hypertension, smoking, lipid-lowering medication, or digoxin, as well as a BMI <30 kg/m^2 and urinary ACR < 3.4 mg/mmol (<30mg/g).

[b]Effective degrees of freedom is related to the complexity of the smoothness of a given variable and can be a decimal number. Higher degrees correspond to a wigglier curve; a degree of 1 corresponds to a linear relationship.

[c]The Akaike Information Criterion (AIC) measures the trade-off between the goodness of fit and the simplicity of a model. Lower values correspond to a better trade-off.

Table S3. The relationship between age, sex, health status, and GFR in a generalized additive mixed models.

	Model 4[a]		
	β	(95 % CI)	P value
Linear effects on baseline GFR			
Intercept, mL/min/1.73 m2	77.6	(72.4 to 82.8)	
Male sex	2.03	(-0.78 to 4.82)	0.16
Being healthy[b]	-3.46	(-5.35 to -1.56)	<0.001
Linear effects on the GFR change rate, mL/min/1.73 m^2/year			
Being healthy	0.24	(0.10 to 0.38)	<0.001
Nonlinear effects	Effective degrees of freedom[b]		
Age (time variable), y	2.40		0.03
Interaction between age and male sex	2.54		<0.001
Aikaike Information Criterion[d]	33838		

[a]Adjusted for sex-specific baseline age, and body-mass index, fasting glucose, and systolic BP as time-dependent continuous variables (including an interaction-term with time for each of them)

[b]Healthy is defined as no cardiovascular disease, cancer, diabetes, hypertension, smoking, lipid-lowering medication or digoxin, BMI <30 kg/m^2 and urinary ACR < 3.4 mg/mmol (<30 mg/g).

[c]Effective degrees of freedom is related to the complexity of the smoothness of a given variable and can be a decimal number. Higher degrees correspond to a wigglier curve; a degree of 1 corresponds to a linear relationship.

[d]The Akaike Information Criterion (AIC) measures the trade-off between the goodness of fit and the simplicity of a model. Lower values correspond to a better trade-off.

Table S4. The relationship between age, sex, health status and absolute GFR in mL/min in generalized additive mixed models.

	β	(95 % CI)	P-value
Linear effects on the baseline GFR			
Intercept, mL/min	92.6	(92.6 to 94.8)	
Male sex	7.17	(3.80 to 10.54)	<4e-5
Being healthy[a]	-3.22	(-5.20 to -1.24)	<0.01
Linear effects on GFR change rate, mL/min/year			
Being healthy[a]	0.18	(0.03 to 0.32)	0.01
Nonlinear effects	Effective degrees of freedom[b]		
Age (time variable), y	1.00		<0.001
Interaction between age and male sex	3.28		<3e-16

The model was adjusted for sex-specific baseline age and time-dependent variables body weight and height and their interaction with time (effect on the slope).

[a]Healthy is defined as no cardiovascular disease, cancer, diabetes, hypertension, smoking, lipid-lowering medication or digoxin, BMI <30 kg/m^2 and ACR >3.4 mg/mmol (<30mg/g).

[b]Effective degrees of freedom are related to the complexity of the smoothness of a given variable and can be a decimal number. Higher degrees correspond to a wigglier curve; a degree of 1 corresponds to a linear relationship.

Table S5. The relationship between age, sex, health status and eGFR in generalized additive mixed models.

	Model 3 eGFRcrea			Model 3 eGFRcys			Model 3 eGFRcyscrea		
	β	(95 % CI)	P value	β	(95 % CI)	P value	β	(95 % CI)	P value
Linear effects on eGFR at baseline									
Intercept, mL/min/1.73 m^2	87.0	(85.6 to 88.4)		85.3	(83.6 to 87.1)		87.4	(84.1 to 87.9)	
Male sex	-0.26	(-2.29 to 1.77)	0.8	3.34	(0.80 to 5.89)	0.01	1.38	(-1.01 to 3.77)	0.3
Being healthy[a]	-1.42	(-2.57 to -0.27)	0.02	0.53	(-0.91 to 1.97)	0.5	-0.63	(-1.92 to 0.64)	0.3
Linear effects on eGFR change rate, mL/min/1.73 m^2/year									
Being healthy[a]	0.11	(0.03 to 0.19)	0.01	0.07	(-0.04 to 0.18)	0.2	0.10	(0.00 to 0.19)	0.04
Nonlinear effects	Effective degrees of freedom[b]			Effective degrees of freedom[b]			Effective degrees of freedom[b]		
Age (time variable), y	1.00		<2e-16	7.20		<2e-16	5.64		<2e-16
Interaction between age and male sex	1.54		0.7	1.00		<0.001	1.00		0.2

All models were adjusted for sex-specific baseline age.

[a]Healthy is defined as no cardiovascular disease, cancer, diabetes, hypertension, smoking, lipid-lowering medication or digoxin, as well as a BMI <30 kg/m^2 and urinary ACR < 3.4 mg/mmol (<30 mg/g). [b]Effective degrees of freedom is related to the complexity of the smooth of a given variable and can be a decimal number. Higher degrees correspond to a wigglier curve; a degree of 1 corresponds to a linear relationship.

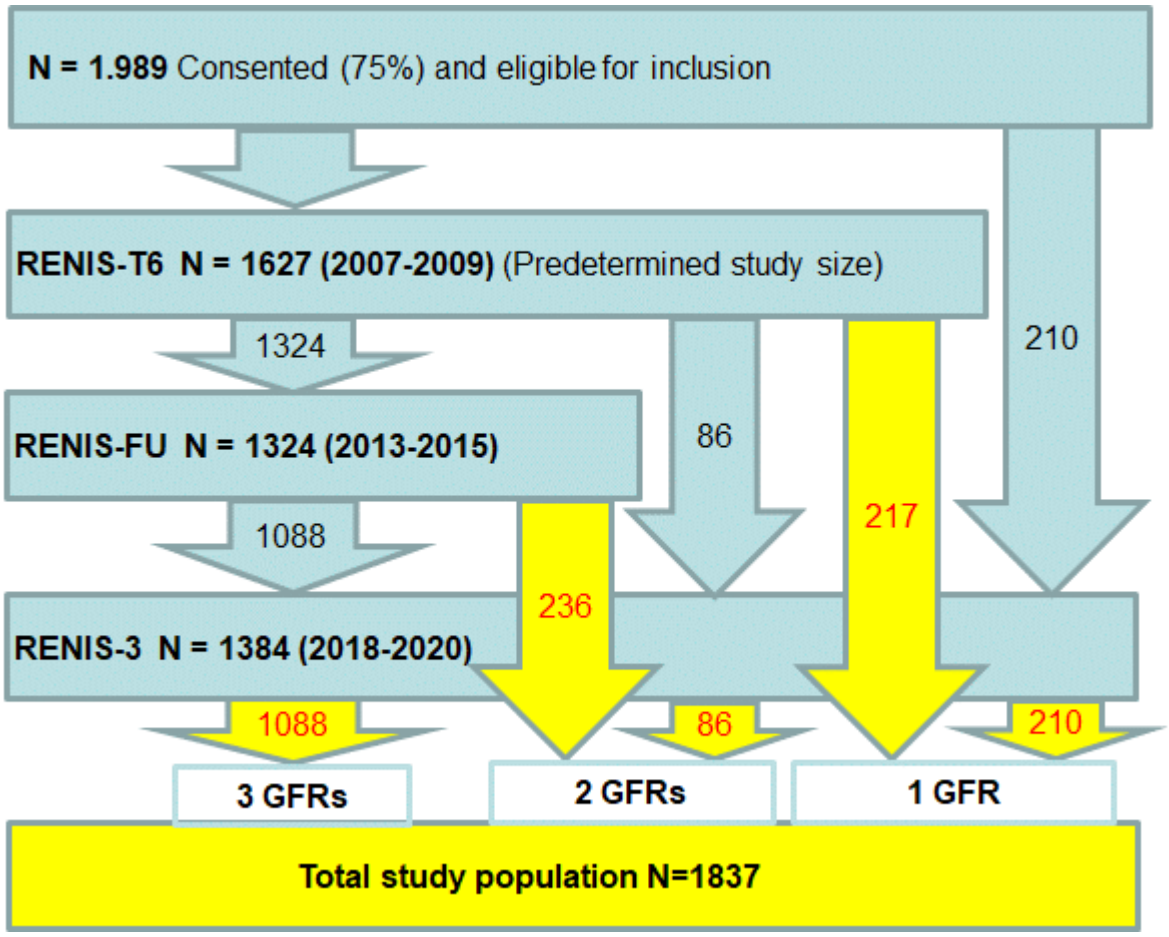

Figure S1. The total study population with at least one GFR measurement in the Renal Iohexol Clearance Survey (RENIS)

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