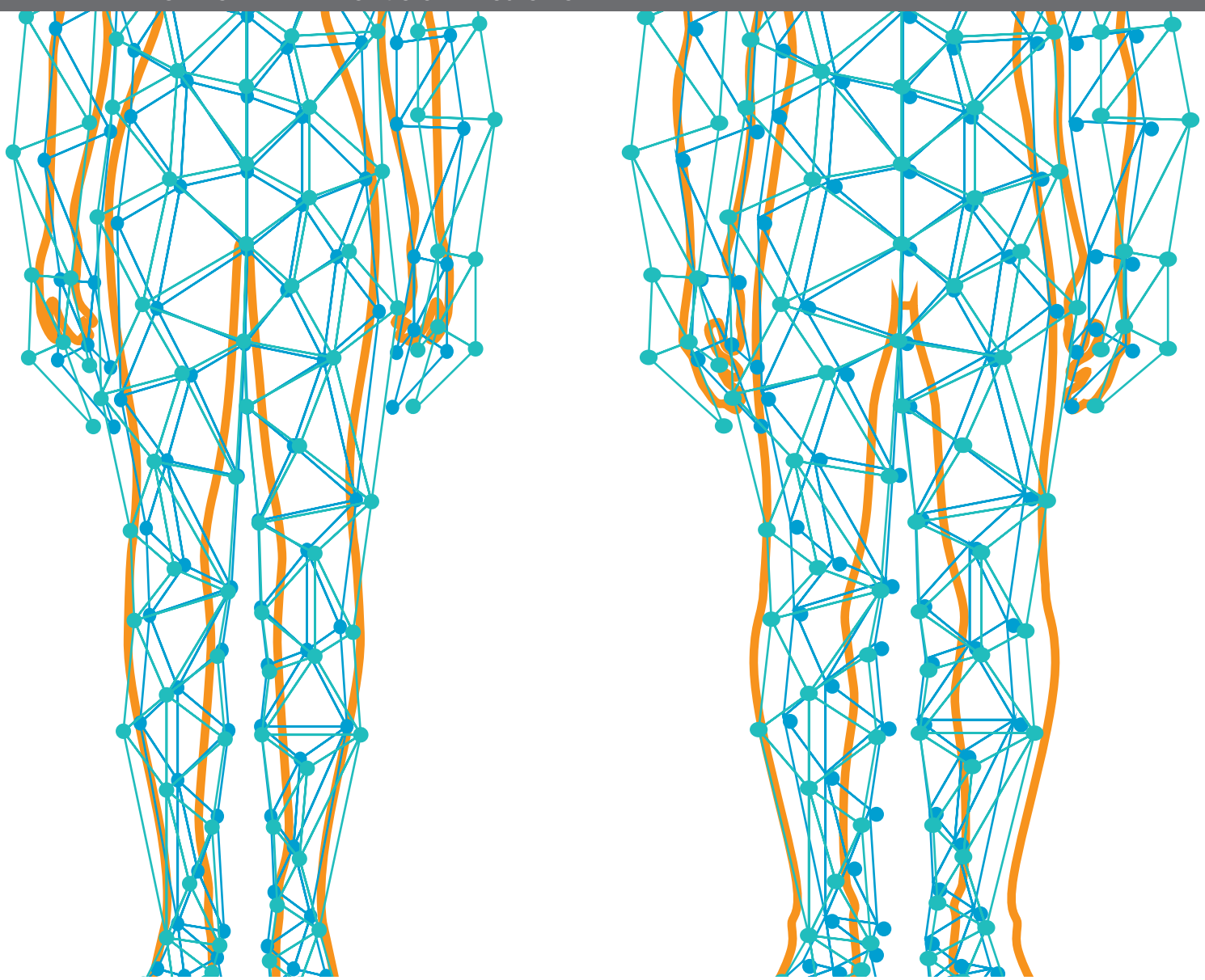
GLOMERULAR FILTRATION RATE IN CHRONIC KIDNEY DISEASE

EDITED BY: Ana Cusumano, Guillermo Rosa Diez and Carmen Tzanno-Martins

PUBLISHED IN: Frontiers in Medicine



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-83250-900-5
DOI 10.3389/978-2-83250-900-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

GLOMERULAR FILTRATION RATE IN CHRONIC KIDNEY DISEASE

Topic Editors:
Ana Cusumano, Norberto Quirno Medical Education and Clinical Research Center (CEMIC), Argentina
Guillermo Rosa Diez, Italian Hospital of Buenos Aires, Argentina
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Citation: Cusumano, A., Diez, G. R., Tzanno-Martins, C., eds. (2023). Glomerular Filtration Rate in Chronic Kidney Disease. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-900-5

Table of Contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 10 January 2023
DOI 10.3389/fmed.2022.1105384

OPEN ACCESS

EDITED AND REVIEWED BY
Minnie M. Sarwal,
University of California, San Francisco, United States

*CORRESPONDENCE
Ana Maria Cusumano
✉ anacusumano@yahoo.com.ar

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 22 November 2022
ACCEPTED 28 December 2022
PUBLISHED 10 January 2023

CITATION
Cusumano AM, Rosa Diez G and Tzanno-Martins C (2023) Editorial: Glomerular filtration rate in Chronic Kidney Disease.
Front. Med. 9:1105384.
doi: 10.3389/fmed.2022.1105384

Editorial: Glomerular filtration rate in Chronic Kidney Disease

Ana Maria Cusumano[1]*, Guillermo Rosa Diez[2] and Carmen Tzanno-Martins[3]

[1]Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Buenos Aires, Argentina, [2]Posgraduate Department, Italian Hospital of Buenos Aires University Institute, Buenos Aires, Argentina, [3]Brazilian Society of Nephrology, São Paulo, Brazil

KEYWORDS

CKD, GFR, glifozins, vitamin D, acidosis, uric acid

Editorial on the Research Topic
Glomerular filtration rate in Chronic Kidney Disease

Chronic Kidney Disease (CKD) is defined as "abnormalities of kidney structure or function, present for more than 3 months, with health implications" and is classified according to the cause, the glomerular filtration rate (GFR) category, and the magnitude of albuminuria (1). So, the diagnosis of CKD progression is based on two key parameters: GFR and the presence and extent of albuminuria. GFR is considered the best global index of renal function since its decrease usually correlates with functional renal mass. GFR can be easily estimated (eGFR) through equations that include endogenous analytes such as creatinine or cystatin C, alone or combined, and anthropometric and demographic factors. At the individual level, the accuracy of an eGFR equation is defined as (p30), which means that around 85% of GFR determinations are within ±30% of mGFR. eGFR should not be used when extreme body composition is present, such as patients with anorexia nervosa, cirrhosis, debilitated elderly, severe obesity, or when there is a need to administer nephrotoxic drugs with a narrow therapeutic option. When necessary, mGFR can be measured using radioisotopes or contrast media.

The understanding that a reliable and consistent GFR estimation (which means reproducibility under the same conditions) is central for the practice of nephrology in particular and medicine in general. Taking into account its limitations, at present, eGFR is not only a powerful tool for identifying CKD, but it has become fundamental for physicians for early detection, clinical diagnosis, monitoring of progression, indication for admission to replacement therapy, calculation of the dose of drugs excreted by the kidney, and in preparation for invasive diagnostic or therapeutic procedures. As an epidemiological tool, eGFR is not only a simple method to estimate the global burden of CKD, but an instrument for identifying risk factors for progression, understanding the epidemiology of kidney disease concerning different social groups (particularly vulnerable ones), and establishing public policies that intend to reduce CKD at the population level. In kidney disease investigation, it is necessary to determine the risks and benefits of new drugs over CKD progression.

This volume includes several updates on different aspects of GFR in CKD. The evolution from the physiologic concept of glomerular filtration until its measurement through GFR and its present use as a clinical and epidemiological health tool; to estimate GFR in different populations and clinical situations, such as in pediatrics, older adults, and kidney donors and in obese patients and individuals born with low birth weight, all are detailed in several manuscripts. There is also a good update on different equations for eGFR at different pediatric and adult ages.

It is known, at least since the beginning of the century, that CKD increases the risk of cardiovascular disease and vice versa, as both diseases not only share some cardiovascular risk factors (2) but because each one is a risk factor for the other. The complex variety of mechanisms leading from vascular injury to CKD and from CKD to vascular injury are described, in particular the two-way path between arterial stiffness and renal dysfunction. About living kidney donors, there is little consensus and no evidence about what is considered an appropriate GFR for accepting someone as a kidney donor. mGFR, though the gold standard, is not available everywhere. A combination of two methods is suggested (for example, creatinine clearance and eGFR).

The volume also includes several research articles.

Once again, it was confirmed that metabolic acidosis, defined as a serum bicarbonate level < 22 mmol/l is an independent risk factor for kidney progression.

Serum uromodulin (sUmod), a biomarker of tubular mass and kidney function, in patients referred for coronary angiography showed a linear increase in all-cause and CV mortality, from the group with high sUmod-high eGFR < Low sUmod-High eGFR < High sUmod-low GFR < low sUmod-low eGFR was found. The conclusion was that sUmod additionally to creatinine or cystatin C enables a more precise risk modeling for all-cause and CV mortality.

Using data from the NANES 2017-2018, the interactive impacts of smoking (a modifiable risk factor) and sleep on kidney function were studied in a cross-sectional study; the results showed that normal sleep duration was a protective and more crucial factor for non-smokers than for smokers.

The study about uric acid and impairment of renal function in non-diabetic hypertensive patients concluded a uric acid level ≥ 7.5 mg/dl is a probable optimal cutoff value for predicting kidney function deterioration.

In older adults with CKD followed longitudinally, the patent of deterioration of GFR and malnourishment predicted mortality and kidney failure.

Finally, two manuscripts focused on the novel effects of two drugs in CKD: Sodium-Glucose Cotransporter 2 inhibitors (SGLT2i) or gliflozins, and vitamin D. About the SGLT2i, the different proposed mechanisms of action which would result in improving cardiac and renal outcomes are described. Briefly: SGLT2i inhibits renal glucose reabsorption at the proximal tubules by blocking the SGLT2 cotransporters; the resulting glycosuria reduces hyperglycemia and improves Hb_{A1c}. The simultaneous increase in sodium excretion reverses the tubule-glomerular feedback, reducing the mechanism of damage of glomerular hyperfiltration and slowing the progression of CKD. Calorie loss from glycosuria results in weight loss, increased insulin sensitivity, enhanced lipid metabolism, and probably lessened lipotoxicity. Metabolism moves toward gluconeogenesis and ketogenesis, two probably protective effects for the heart and the kidneys.

The manuscript Non-classical Vitamin D Actions for Renal Protection aimed to update on CKD-induced alterations in both systemic and local bioactivation of vitamin D and calcitriol actions, and to develop strategies to effectively reduce the progression of CKD, without considering the extent of secondary hyperparathyroidism but attenuating its most potent inducers: systemic inflammation, arterial hypertension, and renal and CV damage. The pathophysiology that causes the reduction mediated by calcitriol/VDR (vitamin D receptor) in the proinflammatory and hypertensive signals not related to the decrease in klotho was analyzed.

Some conclusions emerge from this volume:

- GFR continues to be a powerful tool for the diagnosis and follow-up of CKD patients and as an epidemiology tool.
- Physicians should be alert about clinical situations where persistent hyperfiltration is present as a mechanism of damage (low birth weight, kidney donors, aging, obesity, CKD, etc.).
- Scopes and limitations of eGFR must be known, so as to identify when mGFR should be preferred.
- Kidney function deterioration increases the risk of CV-related death in CKD and other non-communicable chronic diseases.
- New drugs, like gliflozins, will contribute to reducing both the progression of CV disease and CKD, added to angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists.

What can we expect about eGFR in the near future? Probably GFR estimation should advance to population-type specific equations, with the race component resolved. In obesity stages II and III, the research could conclude in original formulas for estimating GFR and resolve the doubt about standardizing or not to body surface area. Finally, the recent description of the shrunken pore syndrome, diagnosed by a ratio $eGFR_{cystatin\ C}/eGFR_{creatinine} < 0.60$ (3), which can happen without proteinuria, reinforces applying both formulas. Future CKD guidelines probably will delineate when the simultaneous use of both equations is needed.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. *KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*. (2012). Available online at: https://kdigo.org/wp-content/uploads/2017/02/KDIGO_2012_CKD_GL.pdf

2. Sarnak MJ, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis.* (2000) 35(4 Suppl. 1): S117–31. doi: 10.1016/s0272-6386(00)70239-3

3. Grub A. Glomerular filtration and shrunken pore syndrome in children and adults. *Acta Paediatr.* (2021) 110:2503–8. doi: 10.1111/apa.15846

frontiers in Medicine

ORIGINAL RESEARCH
published: 29 July 2021
doi: 10.3389/fmed.2021.707588

Metabolic Acidosis Is an Independent Risk Factor of Renal Progression in Korean Chronic Kidney Disease Patients: The KNOW-CKD Study Results

*Hyo Jin Kim[1,2], Hyunjin Ryu[3], Eunjeong Kang[4], Minjung Kang[3], Miyeun Han[5], Sang Heon Song[1,2], Joongyub Lee[6], Ji Yong Jung[7], Kyu-Beck Lee[8], Suah Sung[9], Eun Young Seong[1,2], Curie Ahn[10] and Kook-Hwan Oh[3,11]**

[1] Department of Internal Medicine, Pusan National University Hospital, Busan, South Korea, [2] Biomedical Research Institute, Pusan National University Hospital, Busan, South Korea, [3] Department of Internal Medicine, Seoul National University Hospital, Seoul, South Korea, [4] Department of Internal Medicine, Ewha Womans University Seoul Hospital, Ewha Womans University College of Medicine, Seoul, South Korea, [5] Department of Internal Medicine, Hallym University Hangang Sacred Heart Hospital, Seoul, South Korea, [6] Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, South Korea, [7] Department of Internal Medicine, Gachon University Gil Medical Center, Gachon University College of Medicine, Incheon, South Korea, [8] Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, South Korea, [9] Department of Internal Medicine, Eulji Medical Center, Eulji University, Seoul, South Korea, [10] Department of Internal Medicine, National Medical Center, Seoul, South Korea, [11] Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Fengxiao Bu, West China Fourth Hospital of Sichuan University, China
Roberto Zatz, University of São Paulo, Brazil

***Correspondence:**
Kook-Hwan Oh
khoh@snu.ac.kr

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *10 May 2021*
Accepted: *06 July 2021*
Published: *29 July 2021*

Citation:
Kim HJ, Ryu H, Kang E, Kang M, Han M, Song SH, Lee J, Jung JY, Lee K-B, Sung S, Seong EY, Ahn C and Oh K-H (2021) Metabolic Acidosis Is an Independent Risk Factor of Renal Progression in Korean Chronic Kidney Disease Patients: The KNOW-CKD Study Results. Front. Med. 8:707588. doi: 10.3389/fmed.2021.707588

Background: We aimed to evaluate serum bicarbonate as a risk factor for renal progression, cardiovascular events, and mortality in Korean CKD patients.

Methods: We analyzed 1,808 participants from a Korean CKD cohort whose serum bicarbonate levels were measured at enrollment. Serum bicarbonate levels were categorized as low, lower normal, higher normal, and high (total carbon dioxide <22, 22–26, 26.1–29.9, and ≥30 mmol/L, respectively) groups. Metabolic acidosis was defined as a serum bicarbonate level <22 mmol/L. The primary outcome was renal events defined as doubling of serum creatinine, 50% reduction of eGFR from the baseline values, or development of end-stage kidney disease. The secondary outcome consisted of cardiovascular events and death. In addition, patients whose eGFR values were measured more than three times during the follow-up period were analyzed for eGFR decline. The rapid decline in eGFR was defined as lower than the median value of the eGFR slope.

Results: The mean serum bicarbonate level was 25.7 ± 3.7 mmol/L and 240 (13.2%) patients had metabolic acidosis. During the follow-up period of 55.2 ± 24.1 months, 545 (30.9%) patients developed renal events and 187 (10.6%) patients developed a composite of cardiovascular events and death. After adjustment, the low serum bicarbonate group experienced 1.27 times more renal events than the lower normal bicarbonate group [hazard ratio (HR): 1.27; 95% CI: 1.01–1.60, $P = 0.043$]. There was no significant association between the bicarbonate groups and the composite outcome of cardiovascular events and death. The low bicarbonate group showed a significantly rapid decline in eGFR [odds ratio (OR): 2.12; 95% CI: 1.39–3.22, $P < 0.001$] compared to the lower normal bicarbonate group.

Conclusions: Metabolic acidosis was significantly associated with increased renal events and a rapid decline in renal function in Korean predialysis CKD patients.

Keywords: metabolic acidosis, serum bicarbonate, chronic kidney disease, renal progression, renal function decline

INTRODUCTION

The kidney plays a major role in the maintenance of acid-base balance (1). Therefore, metabolic acidosis is common in cases of decreasing renal function (2). Metabolic acidosis is usually presented as a lowered serum bicarbonate level. In previous studies, when metabolic acidosis was defined as a serum bicarbonate level <22 mmol/L, 2.3–13%, and 19–37% of patients with chronic kidney disease (CKD) stage 3 and 4, respectively, showed metabolic acidosis (3, 4). Metabolic acidosis in CKD has several adverse effects, such as chronic inflammation, bone disease, impaired glucose tolerance, muscle wasting, and possible deleterious consequences of cardiovascular (CV) disease (2, 5).

Metabolic acidosis can also be associated with an accelerated progression of CKD. Acid retention decreases the pH of the renal interstitial and intracellular compartments, causing a rise in the renal levels of angiotensin II and aldosterone, endothelin, ammonia with activation of complement, and proinflammatory cytokines, which are the factors involved in promoting renal fibrosis and injury (6). Previous studies showed that low serum bicarbonate levels were associated with the progression of CKD in outpatients (9% had underlying CKD) (7). Another study showed that low serum bicarbonate levels were associated with the progression of CKD or development of incident CKD in community-living elders (8). The Chronic Renal Insufficiency Cohort (CRIC) study, an observational longitudinal study of US CKD patients, showed that the risk of developing a renal outcome was 3% lower per mEq/L increase in serum bicarbonate (9). Lower serum bicarbonate levels were independently associated with rapid decline in kidney function in non-CKD (10) or CKD patients (11).

Serum bicarbonate levels can also affect CV events and mortality. Both low and high serum bicarbonate levels were associated with increased all-cause mortality in US veterans with moderate and advanced CKD (12). In the CRIC study, metabolic acidosis was correlated with a nominally higher risk of mortality (26%); this result was not statistically significant (13). The risk of heart failure and death was significantly elevated in patients with serum bicarbonate levels >26 mmol/L in the CRIC study (13). There is little data on the long-term clinical outcomes of metabolic acidosis in Korean CKD patients. Therefore, we aimed to investigate the association between metabolic acidosis and renal progression, all-cause mortality, and CV outcomes in CKD patients using data from a large-scale Korean CKD cohort.

METHODS

Study Design and Population

The KoreaN Cohort Study for Outcome in Patients With Chronic Kidney Disease (KNOW-CKD) was a Korean multicenter prospective cohort study that enrolled subjects with CKD stages 1–5 (predialysis) from nine major university-affiliated hospitals in Korea. The detailed study design and methods of the KNOW-CKD have been described previously (14). Among the 2,238 participants registered in the KNOW-CKD between 2011 and 2016, we included 1,808 subjects whose serum bicarbonate levels were obtained at enrollment. The study protocol was approved by the ethical committee of each participating clinical center and the institutional review boards of Seoul National University Hospital (1104-089-359), Seoul National University Bundang Hospital (B-1106/129-008), Yonsei University Severance Hospital (4-2011-0163)„ Kangbuk Samsung Medical Center (KC11OIMI0441), Seoul St. Mary's Hospital (KC11OIMI0441), Gil Hospital (GIRBA2553), Eulji General Hospital (201105-01), Chonnam National University Hospital (CNUH-2011-092), and Pusan Paik Hospital (11–91) in 2011. All study subjects provided written informed consent. The study protocol was in accordance with the principles of the Declaration of Helsinki.

Clinical Data Collection and Laboratory Measurements

Baseline demographic characteristics such as age, sex, body mass index (BMI), cause of CKD, smoking, comorbidities, and laboratory data at enrollment were extracted from an electronic data management system (http://www.phactax.org), with assistance from the Division of Data Management at Seoul National University Medical Research Collaborating Center. Patients with a fasting serum glucose ≥126 mg/dL, a history of diabetes mellitus (DM), or those on anti-diabetic medication were considered to have DM. Patients with a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg, or a history of hypertension (HTN) were considered to be HTN. Patients considered to have CV disease were those with a history of coronary artery disease, cerebrovascular disease, arrhythmia, congestive heart failure, or peripheral vascular disease. The following laboratory variables were measured using a ≥8-h fasting blood sample at each participating laboratory: total carbon dioxide (TCO_2), hemoglobin, uric acid, albumin, total cholesterol, C-reactive protein (CRP), phosphorous, calcium, and intact parathyroid hormone. Serum TCO_2 was considered a surrogate measure of serum bicarbonate (12, 15). Serum creatinine was measured using an isotope dilution mass spectrometry (IDMS)-traceable method (16) at a central laboratory (Lab Genomics, Korea). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (17). CKD stages 1–5 were defined according to the Kidney Disease: Improving Global Outcomes' guidelines (18). Second voided or random urine samples were immediately sent to a central laboratory to

determine the urine creatinine and protein levels. Urinary protein excretion was quantified using urinary protein/creatinine ratio (UPCR, g/g) and urinary albumin/creatinine ratio (UACR, mg/g). Estimated dietary protein intake (eDPI) was calculated using the Maroni–Mitch formula: 6.25 × [urine urea nitrogen (g/day) + 0.03 × body weight (kg)] + proteinuria (g/day) (19), and DPI was calculated by dividing the eDPI by body weight (g/kg/day).

Study Outcomes

The primary outcome was renal events, defined as eGFR halving or development of end-stage kidney disease. End-stage kidney disease was defined as the initiation of renal replacement therapy, including dialysis or renal transplantation. The secondary composite outcome consisted of CV events and all-cause mortality. Patients were followed until March 2019. The eGFR decline during the follow-up period was also analyzed.

Statistical Analyses

Categorical variables were evaluated using the χ^2-test or Fisher's exact test and presented as frequencies and percentages. Continuous variables were analyzed using the analysis of variance or Kruskal–Wallis test. The Kolmogorov–Smirnov test was used to analyze the normality of the distribution of parameters. The results were presented as mean ± standard deviation for variables with normal distribution and the median (interquartile range) for variables with skewed distribution. A log transformation was used to normalize the CRP and proteinuria variables. Participants were categorized into four groups according to their serum bicarbonate levels. Low, lower normal, higher normal, and high TCO_2 values were defined as <22, 22–26, 26.1–29.9, and ≥30 mmol/L, respectively, considering the guidelines for CKD management, previous reports (7, 13, 20), and normal TCO_2 range for clinical laboratory. Metabolic acidosis was defined as a TCO_2 level <22 mmol/L. We used a Cox proportional hazards model with adjustment, including variables that were significant in a univariable analysis or other clinically relevant variables, to analyze the association between the serum bicarbonate levels and study outcomes. The results were presented as hazard ratios (HRs) and 95% confidence intervals (CIs). Patients who were lost to follow-up were censored at the date of the last examination. The rates of renal function decline per year were calculated using the slope of eGFR obtained from a generalized linear mixed model. Only 1,571 (86.9%) patients whose eGFR values were measured more than three times during the follow-up period were included in the eGFR decline analysis. The rapid decline in eGFR was defined as lower than the median value of the eGFR slope. Binary logistic regression analysis was used to identify the risk factors for the rapid decline of renal function. $P < 0.05$ were considered statistically significant. The SPSS statistical software (SPSS version 20.0, IBM Corporation, Armonk, NY, USA) was used for all descriptive and outcome analyses.

RESULTS

Baseline Clinical Characteristics of Subjects

The clinical characteristics of the study subjects at enrollment are shown in **Table 1**. The mean age of the 1,808 patients was 53.6 ± 12.3 years, and 1,111 patients (61.4%) were males. The mean eGFR was 52.8 ± 30.9 mL/min/1.73 m^2. Patients with DM and HTN comprised 34.8 and 95.8% of the participants, respectively. The mean serum bicarbonate level was 25.7 ± 3.7 mmol/L. When stratified into four groups based on the baseline serum bicarbonate levels, we observed that patients in the low serum bicarbonate group were older ($P = 0.014$) and had a higher prevalence of DM ($P = 0.011$), HTN ($P = 0.019$), and preexisting CV disease ($P < 0.001$) compared to the other three groups. The eGFR ($P < 0.001$) was lower and UPCR ($P < 0.001$) and UACR ($P < 0.001$) were higher in the low serum bicarbonate group. DPI was similar among all bicarbonate groups ($P = 0.214$). Members of the low serum bicarbonate group were prescribed more diuretics than those in the other groups ($P = 0.001$).

Distribution of Serum Bicarbonate and Prevalence of Metabolic Acidosis

Figure 1A shows the distribution of serum bicarbonate across CKD stages. Advanced CKD stages were associated with lower serum bicarbonate levels ($P < 0.001$, P for linear trend <0.001; **Figure 1A**), and 240 (13.2%) patients had metabolic acidosis. The prevalence of metabolic acidosis was higher in patients with advanced CKD ($P < 0.001$, P for linear trend <0.001; **Figure 1B**), and it increased rapidly from CKD stage 4; 1.0, 3.9, 5.8, 12.0, 27.6, and 46.4% patients exhibited metabolic acidosis in CKD stages 1, 2, 3a, 3b, 4, and 5, respectively.

Serum Bicarbonate and Renal Events

Table 2 shows the outcome and event rates according to the serum bicarbonate groups. During the follow-up period of 55.2 ± 24.1 months, 545 (30.9%) patients developed renal events. Patients in the low serum bicarbonate group (57.1%) were at a greater risk for development of renal events compared to the other serum bicarbonate groups ($P < 0.001$; **Figure 2**). The Kaplan–Meier curves (**Figure 3**) showed that the low serum bicarbonate group had a significantly higher cumulative incidence of renal events ($P < 0.001$). The multivariable Cox regression analysis showed that the low serum bicarbonate group experienced 1.27 times more renal events than the lower normal serum bicarbonate group (HR: 1.27; 95% CI: 1.01–1.60, $P = 0.043$; **Table 3**). The higher normal (HR: 0.95; 95% CI: 0.74–1.21, $P = 0.675$) and high serum bicarbonate groups (HR: 0.89; 95% CI: 0.59–1.33, $P = 0.575$) did not experience a significant increase in renal events compared to the lower normal serum bicarbonate group.

Serum Bicarbonate and CV Events and All-Cause Mortality

During the follow-up period, 187 (10.6%) patients developed a composite of CV events and death (**Table 2**). Patients in the low TCO_2 group (15.2%) tended to higher composite

TABLE 1 | Clinical characteristics of the study subjects at enrollment, stratified by serum bicarbonate concentration.

Characteristics	Total (N = 1,808)	Serum TCO_2				*P*-value
		Low (<22 mmol/L) (n = 240)	Lower normal (22–26 mmol/L) (n = 760)	Higher normal (26.1–29.9 mmol/L) (n = 565)	High (≥30 mmol/L) (n = 243)	
Age (mean ± SD)	53.6 ± 12.3	54.7 ± 11.7	54.3 ± 12.4	52.4 ± 12.3	52.7 ± 12.4	0.014
Sex, male, *n* (%)	1,111 (61.4)	141 (58.8)	466 (61.3)	345 (61.1)	159 (65.4)	0.492
BMI (kg/m^2)	24.6 ± 3.4	24.0 ± 3.3	24.7 ± 3.4	24.7 ± 3.6	24.7 ± 3.2	0.024
SBP (mmHg)	127.5 ± 15.6	129.0 ± 19.0	127.0 ± 15.3	127.4 ± 15.1	127.6 ± 13.7	0.389
DM, *n* (%)	627 (34.8)	96 (40.0)	283 (37.2)	179 (31.7)	69 (28.8)	0.011
HTN, *n* (%)	1732 (95.8)	235 (97.9)	735 (96.8)	535 (94.7)	227 (93.4)	0.019
Preexisting CV disease, *n* (%)	302 (16.7)	64 (26.7)	128 (16.8)	82 (14.5)	28 (11.5)	<0.001
CAD, *n* (%)	125 (6.9)	28 (11.8)	56 (7.4)	29 (5.1)	12 (4.9)	0.004
Cerebrovascular ds, *n* (%)	119 (6.6)	20 (8.3)	52 (6.8)	37 (6.5)	10 (4.1)	0.297
HF, *n* (%)	31 (1.7)	11 (4.6)	12 (1.6)	6 (1.1)	2 (0.8)	0.002
Arrhythmia, *n* (%)	41 (2.3)	13 (5.4)	9 (1.2)	14 (2.5)	5 (2.1)	0.002
PVD, *n* (%)	69 (3.8)	15 (6.3)	30 (3.9)	18 (3.2)	6 (2.5)	0.126
Cause of CKD						<0.001
DN, *n* (%)	425 (23.5)	68 (28.3)	213 (28.0)	113 (20.0)	31 (12.8)	
Hypertension, *n* (%)	318 (17.6)	54 (22.5)	135 (17.8)	93 (16.5)	36 (14.8)	
GN, *n* (%)	623 (34.5)	76 (31.7)	250 (32.9)	193 (34.2)	104 (42.8)	
PKD, *n* (%)	326 (18.0)	22 (9.2)	116 (15.3)	133 (23.5)	55 (22.6)	
Others, *n* (%)	116 (6.4)	20 (8.3)	46 (6.1)	33 (5.8)	17 (7.0)	
Smoking status, *n* (%)						0.820
Never	979 (54.1)	125 (52.1)	400 (52.6)	316 (55.9)	138 (56.8)	
Former	554 (30.6)	77 (32.1)	239 (31.4)	165 (29.2)	72 (30.0)	
Current	275 (15.2)	38 (15.8)	121 (15.9)	84 (14.9)	32 (13.2)	
TCO_2 (mmol/L)	25.7 ± 3.7	19.6 ± 1.9	24.2 ± 1.3	27.9 ± 0.9	31.3 ± 1.4	<0.001
eGFR (mL/min/1.73m^2)	52.8 ± 30.9	27.7 ± 18.3	45.1 ± 27.7	65.8 ± 29.5	71.9 ± 27.8	<0.001
Hemoglobin (g/dL)	12.8 ± 2.0	11.3 ± 1.7	12.5 ± 2.0	13.5 ± 1.9	13.7 ± 1.8	<0.001
Uric acid (mg/dL)	7.0 ± 1.9	7.5 ± 2.0	7.3 ± 2.0	6.6 ± 1.9	6.6 ± 1.7	<0.001
Albumin (g/dL)	4.2 ± 0.4	4.0 ± 0.4	4.1 ± 0.5	4.3 ± 0.4	4.3 ± 0.4	<0.001
Total cholesterol (mg/dL)	174.3 ± 39.2	164.9 ± 43.8	172.8 ± 39.2	177.6 ± 37.3	180.7 ± 37.1	<0.001
CRP, median, (Q1, Q3) (mg/L)	0.6 (0.2, 1.7)	0.7 (0.4, 2.1)	0.6 (0.2, 1.7)	0.5 (0.2, 1.5)	0.5 (0.2, 1.4)	0.001
Phosphorus (mg/dL)	3.7 ± 0.7	4.1 ± 0.8	3.7 ± 0.7	3.5 ± 0.6	3.6 ± 0.6	<0.001
*Corrected Ca (mg/dL)	9.0 ± 0.4	8.8 ± 0.5	9.0 ± 0.4	9.1 ± 0.4	9.1 ± 0.4	<0.001
iPTH, median (Q1, Q3) (pg/mL)	51.0 (33.5, 83.8)	87.3 (52.9, 146.0)	56.5 (36.7, 94.8)	43.0 (30.1, 64.9)	39.1 (27.6, 53.8)	<0.001
UPCR (Q1, Q3) (g/g)	0.49 (0.15, 1.48)	1.08 (0.39, 2.25)	0.58 (0.19, 1.83)	0.34 (0.10, 0.93)	0.27 (0.07, 0.81)	<0.001
UACR (Q1, Q3) (mg/g)	350 (80, 1052)	767 (238, 1481)	420 (121, 1313)	237 (46, 694)	179 (29, 585)	<0.001
DPI (g/kg/day)	0.96 ± 0.30	0.96 ± 0.39	0.94 ± 0.29	0.98 ± 0.28	0.96 ± 0.25	0.214
Medications						
ACEi or ARB, *n* (%)	1553 (85.9)	209 (87.1)	646 (85.0)	484 (85.7)	214 (88.1)	0.625
Diuretics, *n* (%)	568 (0.3)	95 (0.4)	252 (0.3)	144 (0.3)	77 (0.3)	0.001
†Phosphate binder, *n* (%)	165 (0.1)	32 (0.1)	76 (0.1)	38 (0.1)	19 (0.1)	0.017

P-value represents a statistical difference according to the serum bicarbonate groups of each value using the χ^2-test or Fisher's exact test (categorical variables) or the analysis of variance or Kruskal–Wallis test (continuous variables).

**Corrected Ca (mg/dL) = measured total Ca (mg/dL) + 0.8 × [4 – measured serum albumin (g/dL)].*

†Only calcium-based phosphate binders were prescribed to the members of our patient cohort.

TCO_2, total carbon dioxide; SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DM, diabetes mellitus; HTN, hypertension; CV, cardiovascular; CAD, coronary artery disease; HF, heart failure; PVD, peripheral vascular disease; CKD, chronic kidney disease; DN, diabetic nephropathy; GN, glomerulonephritis; PKD, polycystic kidney disease; eGFR, estimated glomerular filtration rate as determined by the CKD-EPI creatinine equation; CRP, C-reactive protein; Ca, calcium; iPTH, intact parathyroid hormone; UPCR, urine protein creatinine ratio; UACR, urine albumin creatinine ratio; DPI, dietary protein intake; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

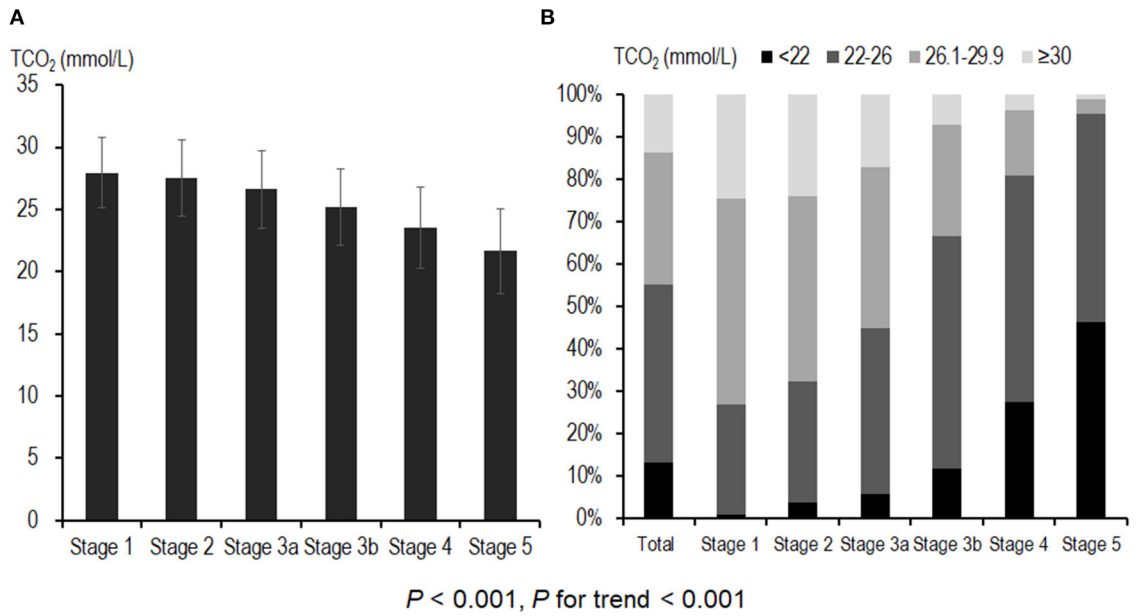


FIGURE 1 | Distribution of serum bicarbonate and prevalence of metabolic acidosis across CKD stages. **(A)** Advanced CKD stages were associated with lower serum bicarbonate levels. A total of 240 (13.2%) patients had metabolic acidosis. **(B)** The prevalence of metabolic acidosis was higher in patients with advanced CKD; 111 (27.6%) and 51 (46.4%) of CKD stage 4 and stage 5 patients, respectively, exhibited metabolic acidosis. CKD, chronic kidney disease; TCO_2, total carbon dioxide.

TABLE 2 | Outcome and event rates according to serum bicarbonate groups.

Outcomes	Overall	Serum TCO_2 group				*P*-value
		Low (<22 mmol/L)	Lower normal (22–26 mmol/L)	Higher normal (26.1–29.9 mmol/L)	High (≥30 mmol/L)	
Number of participants	1,808	240	760	565	243	
Renal events, *n* (%)	545 (30.9)	132 (57.1)	275 (37.0)	104 (18.8)	34 (14.5)	<0.001
eGFR halving, *n* (%)	345 (19.6)	59 (25.5)	176 (23.7)	82 (14.8)	28 (11.9)	<0.001
ESKD, *n* (%)	447 (25.4)	126 (54.5)	231 (31.0)	69 (12.5)	21 (8.9)	<0.001
Composite of CV events and death, *n* (%)	187 (10.6)	35 (15.2)	81 (10.9)	47 (8.5)	24 (10.2)	0.052
Non-fatal CV event, *n* (%)	115 (6.5)	20 (8.7)	47 (6.3)	30 (5.4)	18 (7.7)	0.338
Death, *n* (%)	78 (4.4)	16 (6.9)	37 (5.0)	17 (3.1)	8 (3.4)	0.075

P-value represents a statistical difference according to the serum bicarbonate groups of each event using the χ^2-test.
TCO_2, total carbon dioxide; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; CV, cardiovascular.

secondary outcomes ($P = 0.052$; **Figure 2**). However, in the Cox proportional hazards model after adjustment, there was no significant association between the serum bicarbonate groups and the composite outcome of CV events and death (low vs. lower normal serum bicarbonate group; HR: 1.29; 95% CI: 0.83–2.02, $P = 0.259$; **Supplementary Table 1**).

Association of Serum Bicarbonate With Renal Function Decline

We analyzed renal function decline as the slope of eGFR for 1,571 patients whose eGFR values were measured more than three times during the follow-up period. Mean eGFR slope was -2.48 ± 2.03 mL/min/1.73 m^2/year. The eGFR slope according to serum bicarbonate groups was analyzed. The eGFR slope was lower in the low serum bicarbonate group (-2.93 ± 1.61 mL/min/1.73 m^2/year; **Supplementary Figure 1**). We categorized the patients into two groups according to the median value of the eGFR slope and rapid decline in eGFR was defined as the group with lower than median eGFR. The proportion of rapid decline in eGFR was higher in the low serum bicarbonate group (**Figure 4**). The multivariable binary logistic regression analysis revealed that the low serum bicarbonate group showed a significantly rapid decline in eGFR [odds ratio (OR): 2.12; 95% CI: 1.39–3.22, $P < 0.001$; **Figure 4**] compared to the lower normal serum bicarbonate group.

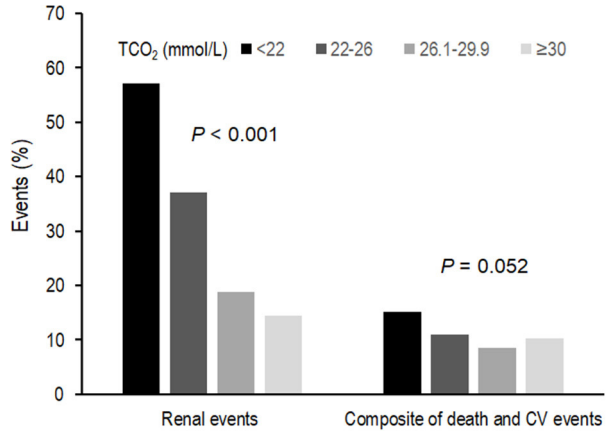


FIGURE 2 | Event rates for renal events and composite of secondary outcomes according to serum bicarbonate groups. Low, lower normal, higher normal, and high TCO_2 values were defined as <22, 22–26, −26.1 to 29.9, and ≥30 mmol/L, respectively. Patients in the low TCO_2 group (57.1%) were at a greater risk of developing renal events compared to the other TCO_2 groups ($P < 0.001$). Patients in the low TCO_2 group (15.2%) tended to higher composite secondary outcomes ($P = 0.052$). TCO_2, total carbon dioxide; CV, cardiovascular.

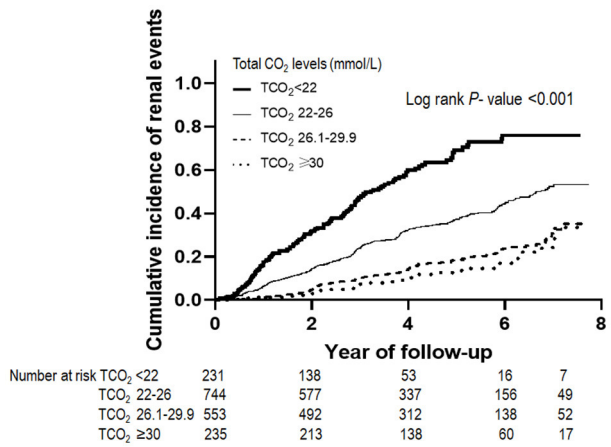


FIGURE 3 | Renal events according to serum bicarbonate groups. The Kaplan–Meier curves show that the low TCO_2 group had a significantly higher cumulative incidence of renal events ($P < 0.001$). TCO_2, total carbon dioxide.

DISCUSSION

In the present study, 240 (13.2%) of the 1,808 CKD patients had metabolic acidosis (TCO_2 <22 mmol/L), and among those with CKD stages 4 and 5, 111 (27.6%) and 51 (46.4%) of patients, respectively, had metabolic acidosis. The incidence of renal events during the follow-up period was higher in patients with metabolic acidosis. Similar to other existing studies, the HR for renal events after adjustment for potential confounders was 1.27 for serum bicarbonate level <22 mmol/L compared to the reference group (serum bicarbonate level 22–26 mmol/L). Patients with metabolic acidosis showed a significantly rapid decline in eGFR. In the present study, there was no significant association between the serum bicarbonate groups and the composite outcome of CV events and death.

Several factors are induced by metabolic acidosis, which cause kidney injury and accelerate the progression of CKD. Kidney damage occurs from acid retention in the interstitial compartment that induces the activation of the renin-angiotensin-aldosterone system and endothelin production (6). In addition, stimulation of complement by increased ammonia production and activation of cytokines also contribute to renal damage. Metabolic acidosis was associated with arterial stiffness in a previous study of our cohort patients (21). Increased arterial stiffness is associated with the risk of renal progression (22); therefore, metabolic acidosis might be associated with CKD progression. Metabolic acidosis is associated with inflammation and oxidative stress, which is associated with renal function deterioration (23, 24); this could also be a mechanistic link between renal injury and metabolic acidosis.

Metabolic acidosis is associated with not only CKD progression but also rapid renal function deterioration. Therefore, proper management is needed to reduce the acid retention. Two modalities that can reduce acid retention include dietary modification to decrease net endogenous acid production or administration of alkali. An experimental study showed that administration of alkali to rats with 5/6 nephrectomy slowed CKD progression and decreased the renal content of endothelin, angiotensin II, and aldosterone (25, 26). A recent randomized trial and other observational studies showed that alkali therapy prevents renal function decline in patients with CKD (27, 28). Large-scale clinical trials are needed to obtain a firm evidence regarding the effectiveness of alkali therapy. Higher serum bicarbonate levels within the normal range were associated with better renal outcomes and survival in the African American Study of Kidney Disease and Hypertension trial (15). Although current guidelines recommend maintaining serum TCO_2 ≥22 mmol/L, according to previous studies on renal outcomes, mortality, or CV outcomes, the ideal target might be 24–26 mmol/L (29). Therefore, further studies are needed to establish optimal serum bicarbonate concentrations.

Serum bicarbonate <22 mmol/L was associated with increased mortality in US veterans (12); however, metabolic acidosis was not significantly associated with mortality in the CRIC study (13). In addition, an association between metabolic acidosis and increased CV event risk independent of eGFR has not yet been clearly identified. The findings of study outcomes may have been slightly different because of the differences in the characteristics of the study population and the reference range of metabolic acidosis. In the present study, we included patients with early CKD (16.3% with CKD stage 1 and 18.5% with CKD stage 2). Furthermore, our study showed that the incidence rates of mortality and CV outcome were lower than those in the CRIC study (30); hence, the results may differ.

The strength of our study is that we included a large number of predialysis CKD patients. However, this study also has several limitations. First, we could not eliminate the potential residual confounders due to the observational nature of the study. Second,

TABLE 3 | Renal events according to serum bicarbonate concentration.

Serum bicarbonate	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Categorical variable								
Low (<22 mmol/L)	2.35 (1.91, 2.90)	<0.001	2.25 (1.82, 2.78)	<0.001	1.31 (1.05, 1.63)	0.018	1.27 (1.01, 1.60)	0.043
Lower normal (22–26 mmol/L)	Reference	–	Reference	–	Reference	–	Reference	–
Higher normal (26.1–29.9 mmol/L)	0.44 (0.35, 0.55)	<0.001	0.45 (0.36, 0.56)	<0.001	0.96 (0.75, 1.22)	0.760	0.95 (0.74, 1.21)	0.675
High (≥30 mmol/L)	0.33 (0.23, 0.47)	<0.001	0.36 (0.25, 0.51)	<0.001	1.03 (0.71, 1.51)	0.863	0.89 (0.59, 1.33)	0.575
Continuous variable								
TCO_2 (per 1 mmol/L increase)	0.83 (0.81, 0.85)	<0.001	0.84 (0.82, 0.86)	<0.001	0.97 (0.94, 1.00)	0.024	0.96 (0.94, 0.99)	0.013

Model 1: Unadjusted.
Model 2: Adjusted for age, sex, HTN, DM, preexisting CVD, systolic blood pressure, BMI.
Model 3: Model 2 + eGFR, log UPCR.
Model 4: Model 3 + albumin, total cholesterol, logCRP, ACEi or ARB use, and diuretics use.
HR, hazard ratio; CI, confidence interval; TCO_2, total carbon dioxide; DM, diabetes mellitus; HTN, hypertension; CVD, cardiovascular disease; BMI, body mass index; eGFR, estimated glomerular filtration rate by CKD-EPI creatinine equation; UPCR, urine protein creatinine ratio; CRP, C-reactive protein; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

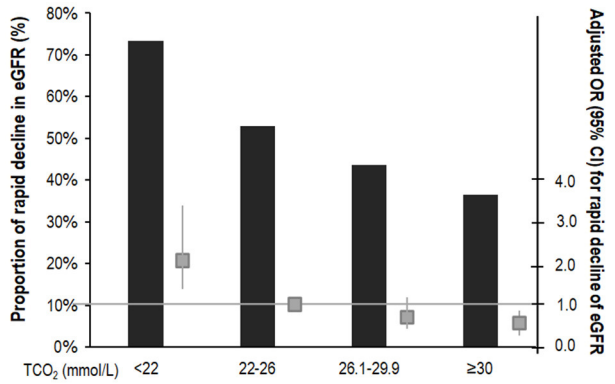


FIGURE 4 | Risk of rapid decline of eGFR according to serum bicarbonate groups. The rapid decline in eGFR was defined as lower than the median value of the eGFR slope. Proportion of the rapid decline of eGFR was higher in the low TCO_2 group. The multivariable binary logistic regression analysis showed that the low TCO_2 group had a significantly rapid decline in eGFR (OR: 2.12; 95% CI: 1.39–3.22, $P < 0.001$) compared to the lower normal TCO_2 group. The column shows a proportion of the rapid decline in eGFR (%). The box plot shows adjusted OR (95% CI) for rapid decline of eGFR. The horizontal solid line represents the reference with an adjusted OR of 1. Adjusted for age, sex, HTN, DM, preexisting CVD, SBP, BMI, eGFR, log UPCR, albumin, total cholesterol, logCRP, ACEi or ARB use, or diuretics. OR, odds ratio; CI, confidence interval; TCO_2, total carbon dioxide; DM, diabetes mellitus; HTN, hypertension; CVD, cardiovascular disease; BMI, body mass index; eGFR, estimated glomerular filtration rate by CKD-EPI creatinine equation; UPCR, urine protein creatinine ratio; CRP, C-reactive protein; ACEi, angiotensin converting enzyme inhibitor; ARB: angiotensin II receptor blocker.

single serum bicarbonate values were used to predict renal events and the composite outcome of mortality or CV events. We did not evaluate the effects of alkali administration (e.g., sodium bicarbonate, sodium lactate, sodium citrate, calcium citrate, etc.) on the incidence of metabolic acidosis and clinical outcomes. There were no patients taking non-calcium-based phosphate binders (e.g., sevelamer carbonate or sevelamer hydrochloride), which affect the acid-base balance. The possibility of dilutional acidosis in metabolic acidosis was excluded because we did not enroll acutely ill patients requiring large volume resuscitation.

CONCLUSION

Metabolic acidosis, defined as a serum bicarbonate level <22 mmol/L, was significantly associated with increased renal events and rapid decline of renal function in this cohort of Korean predialysis CKD patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the study protocol was approved by the ethical committee of each participating clinical center and the institutional review boards of Seoul National University Hospital (1104-089-359), Seoul National University Bundang Hospital (B-1106/129-008), Yonsei University Severance Hospital (4-2011-0163), Kangbuk Samsung Medical Center (2011-01-076), Seoul St. Mary's Hospital (KC11OIMI0441), Gil Hospital (GIRBA2553), Eulji General Hospital (201105-01), Chonnam National University Hospital (CNUH-2011-092), and Pusan Paik Hospital (11–91). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HJK and K-HO were involved with the conception and design of the study. HJK, HR, EK, MK, MH, JYJ, K-BL, SS, CA, and K-HO were involved with patient data collection and acquisition. HJK, SHS, JL, EYS, and K-HO performed the

analysis and interpretation of data. Article draft and revision were carried out by HJK and K-HO. All authors approved the final manuscript.

FUNDING

This study was supported by the Research Program funded by the Korea Center for Disease Control and Prevention (2011E3300300, 2012E3301100, 2013E3301600, 2013E3301601, 2013E3301602, 2016E3300200, 2016E3300201, 2016E3300202, 2019E320100, and 2019E320101). This KNOW-CKD Study Protocol Summary was registered at ClinicalTrials.gov with accession number NCT01630486. The funders had no role in the study design, data collection or analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

The authors gratefully thank the clinical research staff and nurses that participated in the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.707588/full#supplementary-material

REFERENCES

1. Karim Z, Attmane-Elakeb A, Bichara M. Renal handling of NH4+ in relation to the control of acid-base balance by the kidney. *J Nephrol.* (2002) 15(Suppl. 5):S128–34.
2. Kraut JA, Madias NE. Metabolic acidosis of CKD: an update. *Am J Kidney Dis.* (2016) 67:307–17. doi: 10.1053/j.ajkd.2015.08.028
3. Raphael KL, Zhang Y, Ying J, Greene T. Prevalence of and risk factors for reduced serum bicarbonate in chronic kidney disease. *Nephrology.* (2014) 19:648–54. doi: 10.1111/nep.12315
4. Eustace JA, Astor B, Muntner PM, Ikizler TA, Coresh J. Prevalence of acidosis and inflammation and their association with low serum albumin in chronic kidney disease. *Kidney Int.* (2004) 65:1031–40. doi: 10.1111/j.1523-1755.2004.00481.x
5. Kraut JA, Madias NE. Consequences and therapy of the metabolic acidosis of chronic kidney disease. *Pediatr Nephrol.* (2011) 26:19–28. doi: 10.1007/s00467-010-1564-4
6. Kraut JA, Madias NE. Retarding progression of chronic kidney disease: use of modalities that counter acid retention. *Curr Opin Nephrol Hypertens.* (2018) 27:94–101. doi: 10.1097/MNH.0000000000000386
7. Clinical practice guidelines for nutrition in chronic renal failure. K/DOQI, National Kidney Foundation. *Am J Kidney Dis.* (2000). 35:S1–140.
8. Goldenstein L, Driver TH, Fried LF, Rifkin DE, Patel KV, Yenchek RH, et al. Serum bicarbonate concentrations and kidney disease progression in community-living elders: the Health, Aging, and Body Composition (Health ABC) study. *Am J Kidney Dis.* (2014) 64:542–9. doi: 10.1053/j.ajkd.2014.05.009
9. Dobre M, Yang W, Chen J, Drawz P, Hamm LL, Horwitz E, et al. Association of serum bicarbonate with risk of renal and cardiovascular outcomes in CKD: a report from the Chronic Renal Insufficiency Cohort (CRIC) study. *Am J Kidney Dis.* (2013) 62:670–8. doi: 10.1053/j.ajkd.2013.01.017
10. Driver TH, Shlipak MG, Katz R, Goldenstein L, Sarnak MJ, Hoofnagle AN, et al. Low serum bicarbonate and kidney function decline: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis.* (2014) 64:534–41. doi: 10.1053/j.ajkd.2014.05.008
11. Vallet M, Metzger M, Haymann JP, Flamant M, Gauci C, Thervet E, et al. Urinary ammonia and long-term outcomes in chronic kidney disease. *Kidney Int.* (2015) 88:137–45. doi: 10.1038/ki.2015.52
12. Kovesdy CP, Anderson JE, Kalantar-Zadeh K. Association of serum bicarbonate levels with mortality in patients with non-dialysis-dependent CKD. *Nephrol Dial Transplant.* (2009) 24:1232–7. doi: 10.1093/ndt/gfn633
13. Dobre M, Yang W, Pan Q, Appel L, Bellovich K, Chen J, et al. Persistent high serum bicarbonate and the risk of heart failure in patients with chronic kidney disease (CKD): a report from the Chronic Renal Insufficiency Cohort (CRIC) study. *J Am Heart Assoc.* (2015) 4:e001599. doi: 10.1161/JAHA.114.001599
14. Oh KH, Park SK, Park HC, Chin HJ, Chae DW, Choi KH, et al. KNOW-CKD (KoreaN cohort study for outcome in patients with chronic kidney disease): design and methods. *BMC Nephrol.* (2014) 15:80. doi: 10.1186/1471-2369-15-80
15. Raphael KL, Wei G, Baird BC, Greene T, Beddhu S. Higher serum bicarbonate levels within the normal range are associated with better survival and renal outcomes in African Americans. *Kidney Int.* (2011) 79:356–62. doi: 10.1038/ki.2010.388
16. Siekmann L. Determination of creatinine in human serum by isotope dilution-mass spectrometry. Definitive methods in clinical chemistry, IV. *J Clin Chem Clin Biochem.* (1985) 23:137–44.
17. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
18. Chapter 1: Definition classification of CKD. *Kidney Int Suppl.* (2013) 3:19–62. doi: 10.1038/kisup.2012.64
19. Maroni BJ, Steinman TI, Mitch WE. A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int.* (1985) 27:58–65.
20. Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med.* (2013) 158:825–30. doi: 10.7326/0003-4819-158-11-201306040-00007
21. Kim HJ, Kang E, Ryu H, Han M, Lee KB, Kim YS, et al. Metabolic acidosis is associated with pulse wave velocity in chronic kidney disease: results from the KNOW-CKD study. *Sci Rep.* (2019) 9:16139. doi: 10.1038/s41598-019-52499-6
22. Chen SC, Chang JM, Liu WC, Tsai YC, Tsai JC, Hsu PC, et al. Brachial-ankle pulse wave velocity and rate of renal function decline and mortality in chronic kidney disease. *Clin J Am Soc Nephrol.* (2011) 6:724–32. doi: 10.2215/CJN.07700910
23. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubina P, Lahera V, Luno J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int Suppl.* (2008) S4–9. doi: 10.1038/ki.2008.516
24. Rapa SF, Di Iorio BR, Campiglia P, Heidland A, Marzocco S. Inflammation and oxidative stress in chronic kidney disease-potential therapeutic role of minerals, vitamins and plant-derived metabolites. *Int J Mol Sci.* (2019) 21:263. doi: 10.3390/ijms21010263
25. Gadola L, Noboa O, Marquez MN, Rodriguez MJ, Nin N, Boggia J, et al. Calcium citrate ameliorates the progression of chronic renal injury. *Kidney Int.* (2004) 65:1224–30. doi: 10.1111/j.1523-1755.2004.00496.x
26. Phisitkul S, Hacker C, Simoni J, Tran RM, Wesson DE. Dietary protein causes a decline in the glomerular filtration rate of the remnant kidney mediated by metabolic acidosis and endothelin receptors. *Kidney Int.* (2008) 73:192–9. doi: 10.1038/sj.ki.5002647
27. Di Iorio BR, Bellasi A, Raphael KL, Santoro D, Aucella F, Garofano L, et al. Treatment of metabolic acidosis with sodium bicarbonate delays progression of chronic kidney disease: the UBI study. *J Nephrol.* (2019) 32:989–1001. doi: 10.1007/s40620-019-00656-5

28. Chen W, Levy DS, Abramowitz MK. Acid base balance and progression of kidney disease. *Semin Nephrol.* (2019) 39:406–17. doi: 10.1016/j.semnephrol.2019.04.009
29. Raphael KL. Metabolic acidosis in CKD: core curriculum 2019. *Am J Kidney Dis.* (2019) 74:263–75. doi: 10.1053/j.ajkd.2019.01.036
30. Oh KH, Kang M, Kang E, Ryu H, Han SH, Yoo TH, et al. The KNOW-CKD study: what we have learned about chronic kidney diseases. *Kidney Res Clin Pract.* (2020) 39:121–35. doi: 10.23876/j.krcp.20.042

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 08 September 2021
doi: 10.3389/fmed.2021.723546

Combined Use of Serum Uromodulin and eGFR to Estimate Mortality Risk

Babak Yazdani[1†], Graciela E. Delgado[1,2†], Hubert Scharnagl[3], Bernhard K. Krämer[1,2,4], Heinz Drexel[5,6,7,8], Winfried März[1,3,9], Jürgen E. Scherberich[10,11], Andreas Leiherer[5,6,12‡] and Marcus E. Kleber[1,13‡]*

[1] Vth Department of Medicine (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, [2] Center for Preventive Medicine and Digital Health Baden-Württemberg, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany, [3] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria, [4] European Center for Angioscience, Mannheim, Germany, [5] Vorarlberg Institute for Vascular Investigation and Treatment at the Academic Teaching Hospital Feldkirch, Feldkirch, Austria, [6] Private University of the Principality of Liechtenstein, Triesen, Liechtenstein, [7] Drexel University College of Medicine, Philadelphia, PA, United States, [8] Division of Angiology, Swiss Cardiovascular Center, University Hospital of Bern, Bern, Switzerland, [9] Synlab Academy, Synlab Holding Deutschland GmbH, Mannheim, Germany, [10] Klinikum München-Harlaching, Teaching Hospital of the Ludwig-Maximilians University, Munich, Germany, [11] KfH-München Süd, Munich, Germany, [12] Medical Central Laboratories, Feldkirch, Austria, [13] SYNLAB MVZ Humangenetik Mannheim, Mannheim, Germany

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins,
Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Ashish Verma,
Boston University, United States
Swastika Sur,
University of California, San Francisco, United States

***Correspondence:**
Marcus E. Kleber
marcus.kleber@medma.uni-heidelberg.de

[†] These authors have contributed equally to this work and share first authorship

[‡] These authors have contributed equally to this work and share senior authorship

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 10 June 2021
Accepted: 16 August 2021
Published: 08 September 2021

Citation:
Yazdani B, Delgado GE, Scharnagl H, Krämer BK, Drexel H, März W, Scherberich JE, Leiherer A and Kleber ME (2021) Combined Use of Serum Uromodulin and eGFR to Estimate Mortality Risk. Front. Med. 8:723546. doi: 10.3389/fmed.2021.723546

Serum uromodulin (sUmod) shows a strong direct correlation with eGFR in patients with impaired kidney function and an inverse association with mortality. However, there are patients in whom only one of both markers is decreased. Therefore, we aimed to investigate the effect of marker discordance on mortality risk. sUmod and eGFR were available in 3,057 participants of the Ludwigshafen Risk and Cardiovascular Health study and 529 participants of the VIVIT study. Both studies are monocentric prospective studies of patients that had been referred for coronary angiography. Participants were categorized into four groups according to the median values of sUmod (LURIC: 146 ng/ml, VIVIT: 156) and eGFR (LURIC: 84 ml/min/1.73 m^2, VIVIT: 87). In 945 LURIC participants both markers were high (UHGH), in 935 both were low (ULGL), in 589 only eGFR (UHGL), and in 582 only sUmod (ULGH) was low. After balancing the groups for cardiovascular risk factors, hazard ratios (95%CI) for all-cause mortality as compared to UHGH were 2.03 (1.63–2.52), 1.43 (1.13–1.81), and 1.32 (1.03–1.69) for ULGL, UHGL, and ULGH, respectively. In VIVIT, HRs were 3.12 (1.38–7.08), 2.38 (1.01–5.61), and 2.06 (0.81–5.22). Adding uromodulin to risk prediction models that already included eGFR as a covariate slightly increased the Harrell's C and significantly improved the AUC in LURIC. In UHGL patients, hypertension, heart failure and upregulation of the renin-angiotensin-aldosterone-system seem to be the driving forces of disease development, whereas in ULGH patients metabolic disturbances might be key drivers of increased mortality. In conclusion, SUmod/eGFR subgroups mirror distinct metabolic and clinical patterns. Assessing sUmod additionally to creatinine or cystatin C has the potential to allow a more precise risk modeling and might improve risk stratification.

Keywords: EGFR, mortality, chronic kidney disease, Tamm-Horsfall protein, uromodulin

INTRODUCTION

Uromodulin is the most abundant protein in mammalian urine and is also secreted into the blood in small amounts (sUmod). Results from genome-wide association studies linking genetic variation at the *UMOD* locus with estimated glomerular filtration rate (eGFR) (1), chronic kidney disease (CKD) (1–3), arterial hypertension (4) and diabetic nephropathy (5), as well as the recent development of immunoassays to reliably measure sUmod have renewed the scientific interest in this protein. Polymorphisms in the *UMOD* gene explain ∼25% of the 4% variability in eGFR that can be explained by genetic factors so far (6). Expression and secretion of uromodulin are regulated by an intricate network of transcription factors (7). Studies in mice and analyses of genetic variants of uromodulin in humans have shown important roles of nuclear factor 1-beta (HNF1b) and a glucocorticoid response element that is disrupted by the presence of the rare allele of a common uromodulin SNP (8). *In silico* analyses also suggested a possible role of a number of other transcription factors such as GATA3, SP1, SP3, TP53, POU2F1, RARB, RARA, RXRA, SMAD3, RUNX2, and KLF4 (7).

In the case of pathophysiological conditions they act together to rapidly modulate Umod concentration. Due to its immunomodulatory and anti-inflammatory properties, sUmod concentrations might be actively increased in the setting of systemic illnesses to reduce systemic inflammation (9, 10). This might partly explain the consistently observed inverse association with cardiovascular risk (11–13).

A report from the SPRINT trial showed baseline urinary uromodulin to be associated with the primary endpoint of cardiovascular events in patients with an eGFR <60 ml/min/1.73 m^2, independently from eGFR and albuminuria (14). We recently investigated the association of sUmod with mortality in the Ludwigshafen Risk and Cardiovascular Health study (LURIC) and the VIVIT study and found sUmod to be an independent predictor for mortality, even in models adjusted for eGFR in patients with median to high cardiovascular risk (11, 12). Recently, this association has also been confirmed in a population-based study (15). While sUmod showed a strong direct correlation with eGFR there were subgroups of patients in which only one of these markers was decreased. Therefore, we aimed to examine the impact of eGFR/uromodulin discordance on the individual mortality risk and further sought to determine the clinical, biochemical, and genetic characteristics of these subgroups.

METHODS

Subjects

The LURIC study enrolled 3,316 individuals between 1997 and 2000 at the Ludwigshafen Heart Center in South-West Germany (16) and the VIVIT study 1,048 individuals between 2005 and 2008 at the Landeskrankenhaus Feldkirch in the westernmost province of Austria (17). All participants were of European ancestry, and were referred for elective coronary angiography for the evaluation of established or suspected stable CAD. Patients undergoing coronary angiography for other reasons were not enrolled. The ethics committee of the "Landesärztekammer Rheinland-Pfalz" [LURIC, #837.255.97(1394)] and of "Vorarlberg" (VIVIT, EK-2-2013/0008) approved both studies. Both studies were conducted in accordance with the "Declaration of Helsinki." Informed written consent was obtained from all participants. For 3,051 study participants of LURIC and 529 of VIVIT both sUmod and eGFR were available and those were used for further analyses. sUmod and eGFR were measured and calculated using the same blood samples. Information on vital status was obtained from local registries. Death certificates, medical records of local hospitals, and autopsy data were reviewed independently by two experienced clinicians who were blinded to patient characteristics and who classified the causes of death. 917 (30.1%) LURIC participants died during a median follow-up of 9.9 years (8.57–10.7) and 93 VIVIT participants during a median follow-up of 7.3 years (7.0–7.6). Fasting blood samples were obtained by venipuncture at study entry. A summary of analytic methods has been reported previously (16, 18) and detailed information regarding the laboratory measurements, and clinical definitions is provided in the **Supplementary Material**.

Statistical Analyses

Study participants were categorized into tertiles of sUmod/eGFR or into four groups according to the median values of sUmod (146 ng/ml in LURIC and 156 ng/ml in VIVIT) and eGFR (84 ml/min/1.73 m^2 in LURIC and 87 in VIVIT). In LURIC, also clinical cutoff criteria were applied to create the groups using cutpoints of 150 ng/ml for sUmod and 60 ml/min/1.73 m^2 for eGFR. Continuous data are presented as the mean and standard deviation (SD) when normally distributed or as the median and 25th and 75th percentile for non-normally distributed variables. Categorical data are presented as percentages. Statistical differences between groups and continuous variables were determined using ANOVA. Non-normally distributed variables were log-transformed before entering analysis. Tukey's honest significant difference test was used to investigate differences between individual groups using family-wise correction for multiple testing. The chi-square test was used for categorical variables and differences between individual groups were examined using the "chisq.post.hoc" function implemented in the R package "fifer" v1.1 using the false-discovery-rate method for multiple testing correction.

Survival curves for the different groups were calculated by Kaplan-Meier analysis using the R package "survminer" v0.4.3. We also adjusted the distribution of possible confounders by inverse probability weighting, thereby balancing the subgroups for the confounding variables. A weighted Cox model was calculated and we report the result of the robust score test as implemented in the coxph function in R that corresponds to a log-rank test corrected for weighting. The proportional hazard assumption was checked by examination of scaled Schoenfeld residuals. Harrell's C was calculated using the R package hmisc v4.4-1, ROC curves were calculated and compared using the method of Delong as implemented in the R package pROC 1.16.2.

All tests were two-sided and a P-value < 0.05 was considered statistically significant. All analyses were carried out using R

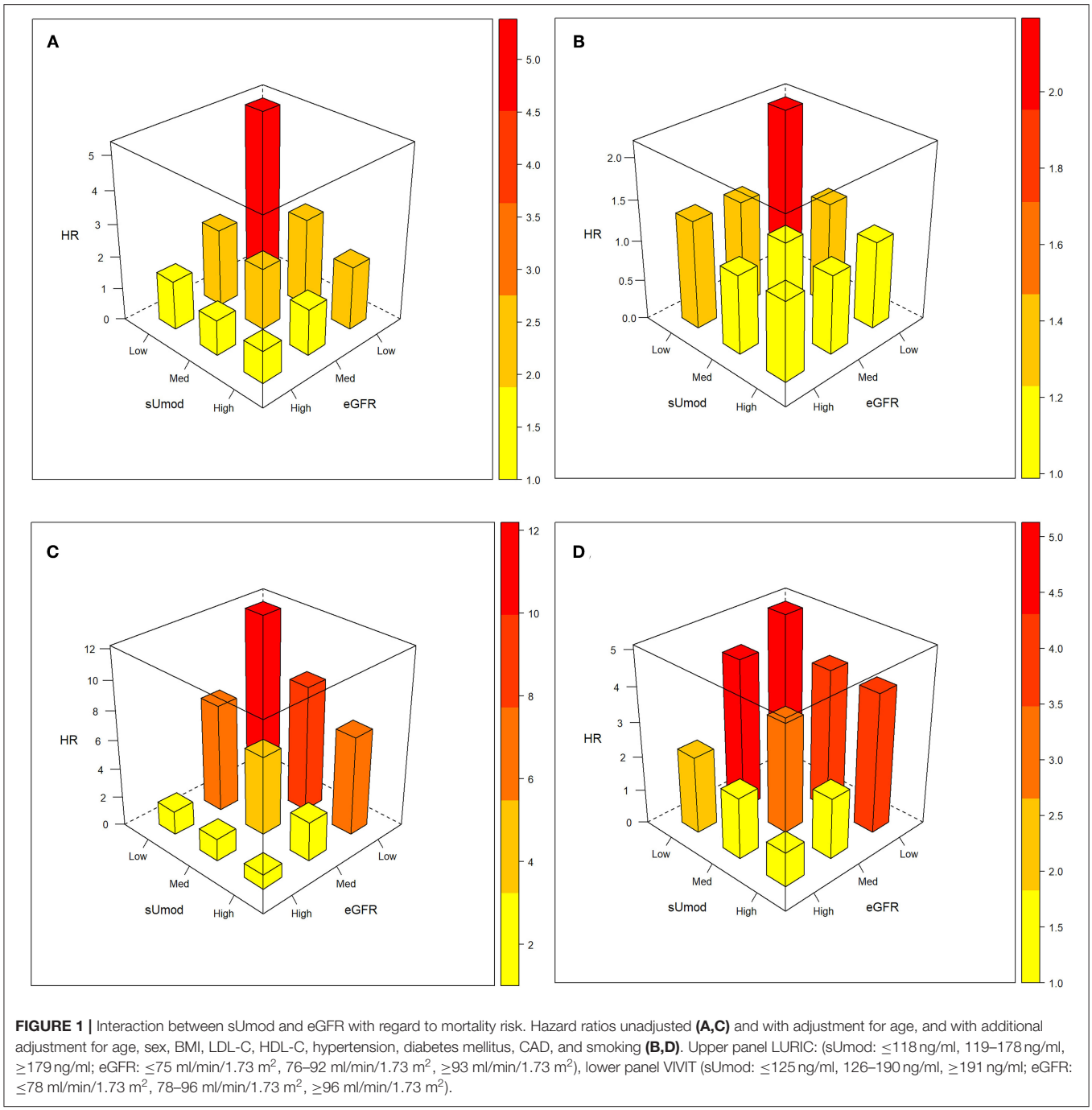


FIGURE 1 | Interaction between sUmod and eGFR with regard to mortality risk. Hazard ratios unadjusted **(A,C)** and with adjustment for age, and with additional adjustment for age, sex, BMI, LDL-C, HDL-C, hypertension, diabetes mellitus, CAD, and smoking **(B,D)**. Upper panel LURIC: (sUmod: ≤118 ng/ml, 119–178 ng/ml, ≥179 ng/ml; eGFR: ≤75 ml/min/1.73 m^2, 76–92 ml/min/1.73 m^2, ≥93 ml/min/1.73 m^2), lower panel VIVIT (sUmod: ≤125 ng/ml, 126–190 ng/ml, ≥191 ng/ml; eGFR: ≤78 ml/min/1.73 m^2, 78–96 ml/min/1.73 m^2, ≥96 ml/min/1.73 m^2).

v4.0.3 [(19) R: A language and environment for statistical computing] (http://www.r-project.org).

RESULTS

Association of eGFR and sUmod With Mortality

Information on both sUmod and eGFR CKD-$EPI_{creat-cys}$ was available for 3,051 participants of the LURIC study and 529 participants of VIVIT. Plots showing the correlation between both biomarkers are shown in **Supplementary Figure 1**.

We stratified our patient cohorts according to tertiles of sUmod and eGFR and calculated hazard ratios (HR) for all-cause mortality for the different combinations (**Figure 1**; **Table 1**). As compared to the reference group with both sUmod and eGFR in the highest tertile, the group with both markers in the lowest tertile had a HR of 5.38 (4.18–6.94) in the unadjusted analysis and a HR of 2.19 (1.65–2.91) after adjustment for age, sex, BMI, LDL-C, HDL-C, coronary artery disease (CAD), hypertension,

TABLE 1 | All-cause mortality according to sUmod and eGFR.

		Unadjusted		Adjusted	
sUmod	**eGFR**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
High	High			1	
High	Medium	1.43 (1.04–1.97)	0.027	0.99 (0.71–1.37)	0.94
		2.68 (0.67–10.73)	0.163	1.77 (0.43–7.25)	0.428
High	Low	1.96 (1.36–2.83)	<0.001	1.10 (0.75–1.61)	0.636
		6.81 (1.76–26.34)	0.005	4.06 (1.00–16.47)	0.05
Medium	High	1.09 (0.77–1.53)	0.635	0.99 (0.70–1.40)	0.956
		1.52 (0.31–7.55)	0.606	1.78 (0.36–8.87)	0.48
Medium	Medium	1.91 (1.41–2.59)	<0.001	1.09 (0.80–1.49)	0.587
		5.48 (1.51–19.91)	0.01	3.23 (0.86–12.15)	0.082
Medium	Low	2.72 (2.04–3.64)	<0.001	1.29 (0.95–1.76)	0.106
		8.72 (2.56–29.76)	0.001	4.10 (1.13–14.92)	0.032
Low	High	1.51 (1.05–2.18)	0.28	1.35 (0.93–1.95)	0.11
		1.64 (0.27–9.79)	0.59	2.24 (0.37–13.47)	0.377
Low	Medium	2.40 (1.77–3.25)	<0.001	1.31 (0.96–1.80)	0.088
		7.45 (2.12–26.14)	0.002	4.41 (1.21–16.00)	0.024
Low	Low	5.38 (4.12–6.94)	<0.001	2.19 (1.65–2.91)	<0.001
		12.20 (3.73–39.85)	<0.001	5.13 (1.44–18.28)	0.012

Unadjusted and adjusted (for age, sex, BMI, LDL-C, HDL-C, smoking, hypertension, CAD, and DM) HRs are summarized for LURIC (upper rows) and VIVIT (lower rows). Green, marker is high; yellow, marker is at medium level; red; marker is low.

diabetes mellitus (DM), and smoking in LURIC. In VIVIT, the HRs were 12.20 (3.73–39.85) and 5.13 (1.44–18.28) for the unadjusted and the adjusted analysis, respectively. Within each eGFR category, the mortality risk increased with lower sUmod. Vice versa, within each sUmod group the mortality risk increased with lower eGFR.

Definition of eGFR/sUmod Subgroups

To further examine the groups with discordant sUmod and eGFR, we stratified our cohort into four groups according to the median sUmod and eGFR values. In LURIC, the median value of sUmod was 146 ng/ml, the median value of eGFR was 84 ml/min/1.72 m^2. In 945 LURIC study participants both markers were above the respective thresholds (UHGH), in 935 both markers were low (ULGL), in 589 only eGFR (UHGL) and in 582 only sUmod (ULGH) was low.

In VIVIT, the median value of sUmod was 156 ng/ml, the median value of eGFR was 87 ml/min/1.72 m^2. The numbers for the different subgroups were 160, 105, 105, and 160 for UHGH, ULGL, UHGL, and ULGH, respectively.

sUmod/eGFR Subgroups and Mortality

Kaplan-Meier analysis revealed a higher mortality risk for UHGL as compared to ULGH (**Figures 2A,B**). As expected, ULGL showed the highest mortality. Adjusted survival curves are shown in **Figures 2C,D**. The distribution of confounding variables (age, sex, BMI, arterial hypertension, CAD, DM, smoking, LDL-C, and HDL-C) in the four groups was balanced by inverse variance weighting. Resulting HR as compared to UHGH were 2.03 (1.63–2.52), 1.43 (1.13–1.81), and 1.32 (1.03–1.69) for ULGL, UHGL, and ULGH in LURIC, respectively. In the VIVIT cohort, HR as compared to UHGH were 3.12 (1.38–7.08), 2.38 (1.01–5.61), and 2.06 (0.81–5.22) for ULGL, UHGL, and ULGH, respectively. Results for cardiovascular mortality were similar (**Supplementary Figure 2**).

We also defined the sUmod/eGFR groups in LURIC according to clinical cutoff criteria: As threshold we chose 60 ml/min/1.73 m^2 for eGFR (to identify those individuals with CKD) and 150 ng/ml for sUmod [approximately below this concentration the mortality risk increases steeply (11)]. The resulting four groups differed substantially in size, especially UHGL was small with only 60 participants, but results were similar (**Supplementary Figure 3**; **Supplementary Table 1**). We also calculated a Cox regression model including an interaction term for sUmod and eGFR and this interaction term was highly significant (**Supplementary Table 1**).

Performance of Uromodulin in Risk Prediction Models

We investigated whether the addition of uromodulin to risk prediction models including eGFR would improve risk prediction. To answer this question, we examined two models: one including age, sex, BMI, CAD, DM, hypertension, smoking, LDL-C, HDL-C, and eGFR and a second model including all covariates and additionally sUmod (**Table 2**). The inclusion of uromodulin slightly increased the Harrell's C and the conventional AUC in both models. The improvement in AUC was statistically significant only in the LURIC cohort. However, a trend toward an improved risk prediction could also be observed in the VIVIT cohort.

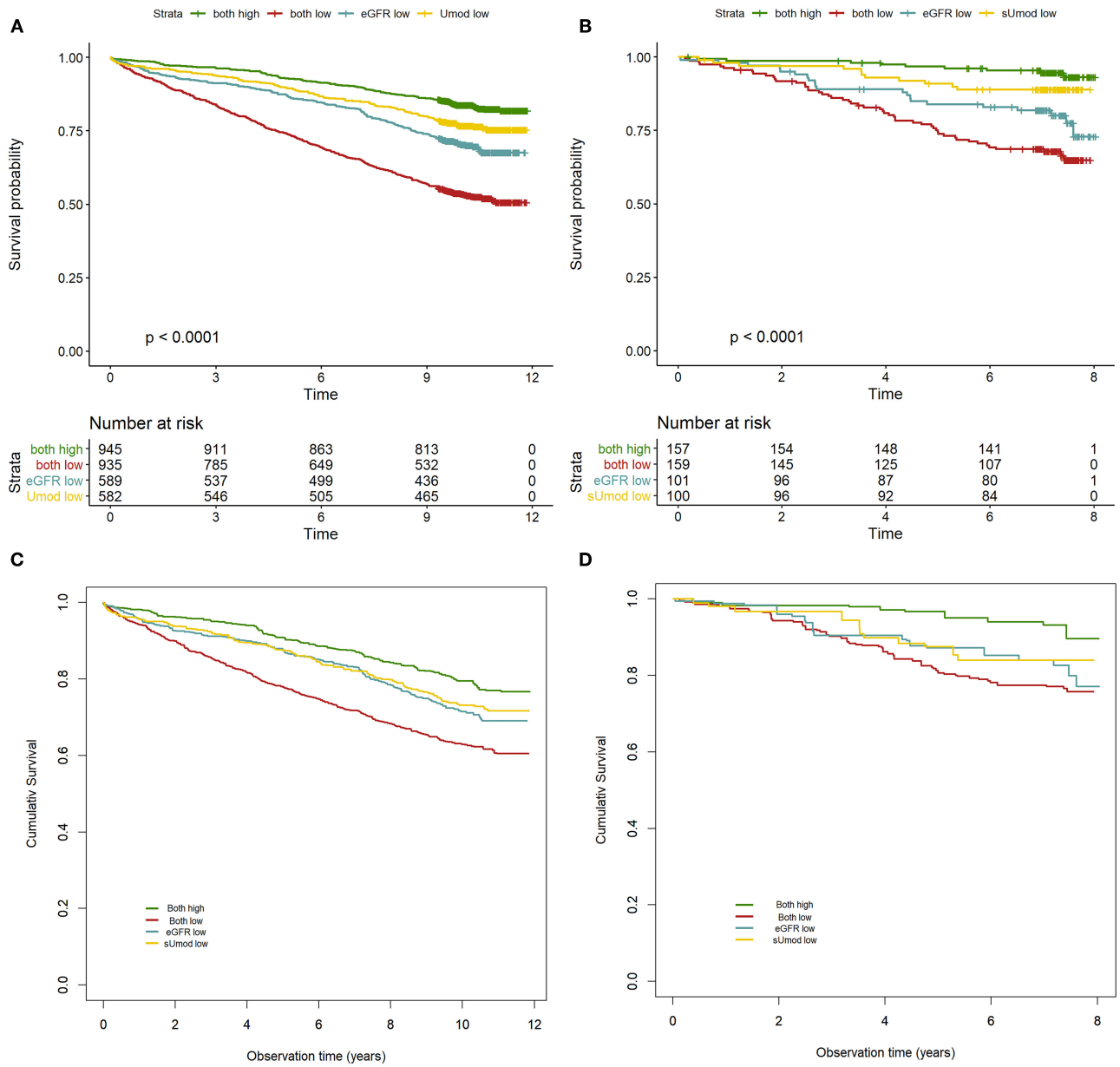


FIGURE 2 | Survival according to eGFR/sUmod groups. Kaplan-Meier survival curves for LURIC and VIVIT **(A,B)** and adjusted survival curves with eGFR/sUmod groups balanced for age, sex, BMI, LDL-C, HDL-C, smoking, hypertension, CAD, and DM by inverse variance weighting for LURIC and VIVIT **(C,D)**. The *P*-value of the robust score test was <0.001 for LURIC and VIVIT.

Characterization of sUmod/eGFR Groups

There were highly significant differences in clinical and biochemical markers between the different sUmod/eGFR groups. In LURIC, comparing UHGH with the groups that had only one marker decreased we observed significantly lower albumin and higher fasting glucose and fatty liver index only in ULGH but not in UHGL (**Table 3**). Contrary, magnesium was only significantly higher in UHGL but not in ULGH, as compared to UHGH. In VIVIT, results were similar for those variables that were available (**Table 4**).

Comparing only UHGL and ULGH, we found that participants in UHGL were older and more often female compared to ULGH. They suffered more often from arterial hypertension and had a tendency toward higher rates of heart failure. Study participants in the groups with low sUmod (ULGL+ULGH) had a higher fatty liver index as compared to the groups with high uromodulin (UHGH+UHGL) but the difference was only significant in LURIC. Looking at differences between UHGL and ULGH groups defined by the clinical cutoff criteria, again

significant associations ($p < 0.001$) were found for the variables age, male gender, heart failure, NTproBNP, and cystatin C (**Supplementary Table 2**). For VIVIT we could not use these criteria due to the low number of samples in the UHGL group ($N = 8$).

TABLE 2 | Risk prediction models for all-cause mortality with and without inclusion of serum uromodulin.

	Harrells C	AUC (95% CI)	P*
Age + sex + eGFR	0.709	0.745 (0.726–0.764)	
	0.748	0.778 (0.729–0.827)	
Age + sex + eGFR + sUmod	0.716	0.752 (0.733–0.771)	0.010
	0.754	0.784 (0.737–0.832)	0.366
Base model	0.718	0.771 (0.753–0.788)	
	0.767	0.799 (0.751–0.847)	
Base model + sUmod	0.722	0.774 (0.757–0.792)	0.043
	0.775	0.808 (0.762–0.854)	0.155

**Model including uromodulin vs. preceding model; base model includes age, sex, BMI, CAD, diabetes mellitus, hypertension, smoking, LDL-C, HDL-C, and eGFR. Upper rows: LURIC, lower rows VIVIT.*

DISCUSSION

Main Findings

Despite a highly significant, direct association of sUmod and eGFR we detected subgroups of patients in whom only one of these biomarkers was decreased. Regarding all-cause and cardiovascular mortality, there was an almost linear increase in mortality risk from UHGH < ULGH < UHGL < ULGL. These findings suggest, that uromodulin modulates mortality risk in patients with both impaired and normal renal function in the way that high uromodulin is associated with higher patient survival. Conversely, in patients with both high and low uromodulin, low eGFR is each time associated with inferior patient survival. When comparing the impact of low eGFR and low uromodulin on all-cause and cardiovascular mortality, the impact of low eGFR appeared to be somewhat stronger than the effect of uromodulin. Adding sUmod to risk prediction models already

TABLE 3 | Characteristics of LURIC study participants according to serum uromodulin (<146 or ≥146 ng/mL) and eGFR (<84 or ≥84 mL/min/1.73 m^2) groups.

Variable	Both high	Both low	eGFR low	sUmod low	P^*_{ANOVA}	$P_{post\text{-}hoc}$		
	UHGH	ULGL	UHGL	ULGH		UHGH vs. UHGL	UHGH vs. ULGH	UHGL vs. ULGH
Age (years)	57(9.76)	68.2(8.69)	67(8.03)	58.7(10.3)	<0.001	<0.001	<0.001	0.003
Male sex (%)	74.6	65.8	57.7	80.4	<0.001	<0.001	<0.001	0.009
BMI (kg/m^2)	27(3.84)	27.7(4.13)	27.7(4.35)	27.7(4.12)	<0.001	1	0.011	0.007
LDL-C (mg/dl)	119(34.8)	113(34.3)	117(34.7)	116(32.4)	0.001	0.88	0.691	0.221
HDL-C (mg/dl)	40(10.9)	36.8(10.5)	39.9(10.9)	38.3(10.8)	<0.001	0.055	1	0.022
TG (mg/dl)	139(103–188)	154(116–208)	144(106–195)	151(113–215)	<0.001	0.024	0.746	<0.001
systolic BP (mmHg)	137(22.2)	145(24.8)	143(23.8)	140(22.1)	<0.001	0.032	<0.001	0.102
diastolic BP (mmHg)	81.1(11.1)	80.6(11.7)	81.5(11.8)	81.1(10.8)	0.482	0.948	0.935	1
Magnesium (mmol/l)	0.847(0.091)	0.862(0.103)	0.859(0.0945)	0.834(0.0879)	<0.001	<0.001	0.082	0.042
Fasting glucose (mg/dl)	99.7(92.1–110)	105(95.2–126)	102(93.8–117)	104(94.7–122)	<0.001	0.272	0.002	<0.001
hsCRP (mg/l)	2.28(1–6.33)	5.25(2.12–10.7)	3.03(1.45–8.3)	3.06(1.22–7.69)	<0.001	0.619	<0.001	0.006
NT-proBNP (ng/ml)	160(68–420)	679(248–1,860)	375(147–1,010)	175(76–486)	<0.001	<0.001	<0.001	0.289
Renin (pg/ml)	17(9–34)	23.5(12–59)	18(9–41)	18(10–35)	<0.001	0.931	0.227	0.61
Angiotensin II (ng/L)	20(12–35)	20(13–34)	20(13–35)	19(12–34)	0.152	0.813	0.45	0.965
Noradrenalin (ng/l)	276(199–380)	339(232–500)	334(233–477)	281(199–394)	<0.001	<0.001	<0.001	0.999
Albumin (g/dl)	4.48(0.535)	4.28(0.553)	4.39(0.524)	4.38(0.573)	<0.001	0.999	0.008	0.007
GOT (U/l)	11.8(7.49)	11.7(7.4)	12.5(9.94)	11.7(6.77)	0.251	0.276	0.338	0.988
FV (U/dl)	112(20.9)	113(23)	114(20.1)	115(21.9)	0.368	0.895	0.829	0.288
Fatty liver index	47.4(25.2–71.4)	58(36.6–77.2)	53.9(31.5–74.3)	55.3(32.1–79.1)	<0.001	0.476	0.006	<0.001
eGFR (ml/min/1.73 m^2)	98.3(9.42)	62(16.2)	72(9.75)	96.5(9.23)	<0.001	<0.001	<0.001	0.022
Uromodulin (ng/ml)	222(62.6)	95.4(31.4)	202(52.4)	107(28.1)	<0.001	<0.001	<0.001	<0.001
CystatinC (mg/l)	0.8(0.74–0.86)	1.12(1–1.37)	1.01(0.93–1.11)	0.83(0.76–0.89)	<0.001	<0.001	<0.001	0.031
Diabetes mellitus (%)	28.1	51.9	41.1	39	<0.001	0.475	<0.001	<0.001
Coronary artery disease (%)	71.7	83.6	76.1	80.2	<0.001	0.097	0.097	0.001
Heart failure (%)	21.8	47.5	35.1	26.1	<0.001	0.001	<0.001	0.054
Hypertension (%)	62.4	81	77.8	70.3	<0.001	0.005	0	0.003
Smoking (active/ex/never, %)	28.4/38.9/32.7	17.6/45.9/36.5	15.3/37.7/47	31.3/40.5/28.2	<0.001	<0.001	<0.001	0.161

**P-value adjusted for multiple testing using the false-discovery-rate method; GOT, glutamate oxalacetate transaminase; FV, factor V; NT-proBNP, N-terminal pro brain natriuretic peptide; TG, triglycerides.*

TABLE 4 | Characteristics of VIVIT study participants according to serum uromodulin (<156 or156 ≥ ng/mL) and eGFR (<87 or ≥87 mL/min/1.73 m^2) groups.

Variable	Both high UHGH	Both low ULGL	sUmod high, eGFR low UHGL	sUmod low, eGFR high ULGH	P_{ANOVA}	$P_{post\text{-}hoc}$		
						UHGH vs. UHGL	UHGH vs. ULGH	UHGL vs. ULGH
N	160	160	104	105				
Age (years)	59(10)	72(8.4)	69(8.4)	59(11)	<0.001	<0.001	1	<0.001
Male sex (%)	71	63	49	72	0.001	0.001	0.998	0.002
BMI (kg/m^2)	28(4)	28(5)	27(4)	28(4)	0.125	0.301	0.976	0.203
LDL-C (mg/dl)	134(38.6)	126(42.1)	131(38.5)	129(38.4)	0.426	0.951	0.763	0.978
HDL-C (mg/dl)	58(18)	58(16)	61(16)	57(15)	0.251	0.421	0.945	0.232
TG (mg/dl)	123(86.3–175)	112(81.3–148)	107(78.0–145)	112(85–175)	0.068	0.080	0.996	0.199
systolic BP (mmHg)	135(17.2)	139(18.7)	139(16.9)	131(18.3)	0.006	0.447	0.345	0.023
diastolic BP (mmHg)	82(10.3)	83(10.8)	83(9.2)	82(9.8)	0.986	0.997	0.999	0.991
Fasting glucose (mg/dl)	98(90.0–1,113)	104(92.0–122)	97(87.0–112)	101(93.5–116)	0.049	0.740	0.750	0.260
CRP (mg/dl)	0.21(0.10–0.39)	0.31(0.16–0.57)	0.19(0.10–0.39)	0.22(0.10–0.47)	0.002	0.961	0.986	0.873
NT-proBNP (ng/ml)	124(64.8–265)	573(160–1,653)	255(172–1,053)	85.5(40.0–276)	<0.001	<0.001	0.716	<0.001
Total protein (g/dl)	7.25(0.50)	7.27(0.47)	7.24(0.46)	7.34(0.36)	0.346	0.995	0.401	0.361
GOT (U/l)	25(21–32)	25(21–29)	25(21–30)	24(21–30)	0.219	1.000	0.766	0.837
Fatty liver index	56.3(25.8)	57.3(26.3)	51.0(26.8)	59.3(27.5)	0.154	0.433	0.814	0.134
eGFR (ml/min/1.73 m^2)	102(9.0)	65(16)	75(9)	101(9)	<0.001	<0.001	0.937	<0.001
Uromodulin (ng/ml)	229(68.8)	102(32.0)	215(56.5)	114(28.1)	<0.001	0.126	<0.001	<0.001
Cystatin C (mg/l)	0.78(0.69–0.83)	1.10(0.98–1.26)	0.98(0.91–1.06)	0.78(0.72–0.84)	<0.001	<0.001	0.780	<0.001
Diabetes mellitus (%)	23	34	22	31	0.051	1	0.484	0.527
Coronary artery disease (%)	80	82	76	80	0.682	0.986	0.873	0.748
Hypertension (%)	84	92	89	84	0.092	0.527	1	0.613
Smoking (active/ex/never, %)	23/45/32	11/46/43	14/36/50	21/46/33	0.024	0.259	0.968	0.594

including eGFR significantly improved risk prediction in LURIC. Furthermore, when comparing UHGL with ULGH, we found significant differences in age, sex, NT-proBNP, and prevalent heart failure.

So far, uromodulin is the only known kidney-specific protein (that can also be measured in the blood) and it has been suggested to serve as a biomarker for nephron mass (20) or the renal functional reserve. Recently, Pruijm et al. reported a strong correlation between 24-h uromodulin excretion with kidney length and volume determined by renal ultrasonography as well as an association with markers of tubular function in a population-based cohort (21) and conclude that uromodulin "may reflect tubule activity in the general population." Indeed, mutations in the uromodulin gene give rise to autosomal dominant tubulointerstitial kidney disease uromodulin-related (ADTKD-UMOD) and reduced uromodulin excretion has been reported for a number of renal diseases, e.g., glomerulonephritis, diabetic nephropathy, or IgA nephropathy (6, 22–25). In IgA nephropathy, uromodulin was shown to be associated with interstitial fibrosis/tubular atrophy and to contribute to eGFR decline (24). A report from the SPRINT trial showed that lower uromodulin in urine was associated with incident acute kidney injury, independent of eGFR and albuminuria (26). On the other hand, in healthy kidney donors no correlation between serum uromodulin and eGFR has been observed (27). In addition, large GWAS metaanalyses have identified polymorphisms in the UMOD promoter that are associated with a lower concentration of uromodulin but higher eGFR (1), a lower risk for CKD, lower blood pressure, a lower risk of hypertension (4), and left atrial remodeling (28). This is explained by the fact that uromodulin increases the activity of ion channels like the NaKCC2 ion transporter or ROMK in the TAL and thereby enhances salt uptake, which then leads to higher blood pressure and increased cardiorenal risk.

It has been suggested that the link between uromodulin and NaKCC2 is mediated through the regulation of intracellular chloride levels and modulation of the chloride sensitive WNK-SPAK/OSR1 pathway (29, 30), which leads to an increased phosphorylation of NKCC2. The mechanism by which uromodulin modulates SPAK/OSR1 activity could involve potentialization of with-no-lysine kinase (WNK) activity, as previously suggested (29).

NKCC2 and uromodulin are distributed in close spatial vicinity on the surface of TAL cells and both share the same lipid raft localization (31) and so it has been proposed that uromodulin might act as a scaffold for WNK-SPAK/OSR1-dependent activation of NKCC2. Recently it has been demonstrated that the activity of this pathway is increased in the kidneys of mice

lacking hepsin, a transmembrane serine protease, which is critical for the luminal release of uromodulin (32). The authors could also show that this goes along with an increased phosphorylation of NKCC2.

It has been shown that Umod knockout mice are resistant to salt-induced changes in blood pressure and a transcriptome study reported that Umod is necessary for the upregulation of heat shock transcripts and the transcripts of seven electrolyte transporters in response to salt stress (33). Further, it has recently been reported that uromodulin also plays a role in the reabsorption of NaCl and the fine-tuning of urinary calcium and magnesium excretion in the distal convoluted tubule (34, 35). In our study, the concentration of magnesium was significantly higher in the groups with low sUmod in LURIC. Another explanation for the seemingly paradoxical association of Umod promoter variants with lower uromodulin but higher eGFR proposes that the effect of these SNPs on kidney risk is independent of Umod expression but due to effects on the expression of neighboring or distal genes that are involved in kidney disease (10).

In large proportions of our cohorts, we observed a discordant behavior between sUmod and eGFR (38% in LURIC, 40% in VIVIT). Regarding ULGH it has been shown that lower concentrations of sUmod may be a novel marker to identify early kidney function loss even when serum creatinine values are still within a normal range (36). The synthesis of Umod per functioning remaining nephron unit is increased in patients with CKD, but may not compensate the overall loss of renal parenchyma (37). In a similar manner, quantitative enzyme and immunohistological analyses of kidney tissue sections from patients with endstage CKD disclosed upregulation of various proteins in single "resistant" hypertrophic and hypermetabolic nephrons (38). Although uromodulin synthesis and intracellular protein transfer might not be disturbed in these surviving nephrons, preinjured epithelia of the thick ascending limb of Henle (TAL) segment should downmodulate uromodulin secretion at a very early stage. This thesis is supported by the observation that sUmod levels decline in early CKD where creatinine and cystatin c concentrations still remain unchanged (39).

This proposed state of early renal damage, with a mean eGFR difference of 25 ml/min/1.73 m^2 in LURIC and 26 ml/min/1.73 m^2 in VIVIT, may partly explain the higher mortality in ULGH vs. UHGH patients groups. ULGH patients might presumably also display a more rapid CKD progression; however this has not been studied in LURIC. In VIVIT, serum creatinine has been measured at study baseline and at a 4-year follow-up. Creatinine increased stronger in the ULGH group (0.78–0.83; paired *t*-test $p = 0.002$) as compared to the UHGL group (0.89–0.92; $p = 0.159$).

Regarding the survival advantage of the ULGH group compared to the ULGL group, the main cause probably is renal function, that is on average at an eGFR of 62 mL/min/1.73 m^2 and is considerably higher as compared to ULGL, despite an early, pre-clinical renal damage [when compared to UHGH].

Importantly, rates of hypertension and heart failure are higher in UHGL than in ULGH in LURIC, suggesting that sodium and fluid overload occur that relate to high sUmod and cannot be balanced by a largely preserved renal function such as in the ULGH patient group.

In VIVIT, there was no significant difference in the rates of hypertension between both groups. Information on heart failure was not available in this cohort but the concentration of NT-proBNP was almost three times higher in the ULGH group.

Conversely, when analyzing the higher mortality in UHGL vs. UHGH patients groups, a difference in mortality that is exceeding the difference between ULGH vs. UHGH by far, the obvious culprit is impaired renal function, that associates with higher rates of arterial hypertension, heart failure, and DM. The survival advantage when comparing UHGL with ULGL may be explained by protective, i.e., antiinflammatory and immunomodulatory effects of normal sUmod levels and by a somewhat better renal function. The difference in eGFR between both groups was 10 ml/min/1.73 m^2 in favor of the UHGL patient group.

Applying a stricter clinical cutoff of 60 ml/min/1.73 m^2 for eGFR in LURIC, we found significant differences between UHGL and ULGH regarding a number of vasoactive biomarkers that were not apparent in the analyses using the median groups (**Supplementary Table 1**). NT-proBNP and angiotensin II were significantly higher in UHGL as compared to ULGH. It is important to notice that the percentage of patients suffering from heart failure and hypertension was higher in UHGL, possibly due to a sUmod mediated enhanced tubular activation of the NKCL-cotransporter. It is tempting to speculate that elevated central venous pressure in heart failure might promote renal congestion (40). A reduced kidney perfusion pressure, associated with potential medullary hypoxia, may lead to an activation of the sympathetic nervous system and an upregulation of the renin-angiotensin-aldosterone (RAAS) system, which helps to explain the elevated concentrations of renin, angiotensin II and noradrenaline in UHGL. Increased concentrations of angiotensin-II and catecholamines consecutively promote glomerular arteriolar vasoconstriction, thereby decreasing renal plasma flow and ultimately eGFR (41). On the other hand, patients in the ULGH group showed higher triglycerides, lower mean LDL particle radius, higher fatty liver index and a higher prevalence of CAD as compared to UHGL.

Of note, a recent study found that sUmod function might be impaired by carbamylation in the setting of CKD (42) and that could also partly account for the higher mortality in the UHGL group as compared to ULGH.

Strengths and Limitations

All LURIC and VIVIT Study participants were of European origin, therefore our findings may not be applicable to other ethnicities. Both studies recruited participants that had been referred for coronary angiography. Our results may therefore not be generalizable to a healthy population. Uromodulin was only measured once in baseline samples. Urine samples were not available to assess urinary uromodulin and eGFR was only calculated once at baseline in LURIC.

The major strengths of our cohorts are, however, the precise clinical and metabolic characterization of the participants, including the availability of coronary angiograms, their cross-sectional and prospective design, and the long-term follow-up.

CONCLUSION

In conclusion, assessing sUmod additionally to creatinine or cystatin C allows a more precise risk modeling for all-cause and cardiovascular mortality and might potentially improve risk stratification. Patients in whom only eGFR is decreased but sUmod remains high are more likely to be male and suffering from heart failure, possibly due to a sUmod mediated enhanced tubular activation of the NKCL-cotransporter. In patients in whom only sUmod is decreased but eGFR remains high we observed a higher prevalence of CAD, a higher fatty liver index and elevated triglycerides that might point to metabolic disturbances as key drivers of increased mortality. The joint consideration of eGFR and uromodulin may have the potential to dissect different forms of cardiorenal syndromes according to their main pathophysiological drivers. Distinct intrinsic metabolic and congruent clinical patterns related to sUmod/eGFR profiles as addressed here for the first time may also govern the susceptibility to risk indicators for kidney health and disease.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Due to the articles of Ludwigshafen Risk and Cardiovascular Health (LURIC) Study gGmbH, which needs to acknowledge the German Data Protection Act and the consent given by the study participants, data cannot be released to the public domain. The exploitation of the (LURIC) Study database is governed by the articles of the LURIC Study GmbH (non-profit LLC), registered under number HRB 7668 at the commercial registry of Freiburg in Breisgau, Germany. According to the articles of the organization, data access is made available to researchers upon request and approval. This procedure makes sure that rules of good scientific practice are followed and that credit is given to the people who have been in charge of the design and the organization of the study. Interested researchers are invited to address their request or proposal to Kai Grunwald (Kai.Grunwald@weitnauer.net) or to the Principal Investigator of the LURIC Study, Winfried März (winfried.maerz@luric-online.de) who are in charge of supervising ethical and legal aspects of the LURIC study. Finally, the authors confirm that they accessed these data upon approval by LURIC and that all other researchers can access the data upon approval in the same manner the authors did. Similarly, the data from VIVIT cannot be released to the public domain due to Austrian Data Protection Act, but access is also made available to researchers upon request and approval (Contact: Heinz Drexel; vivit@lkhf.at). Requests to access these datasets should be directed to Heinz Drexel; vivit@lkhf.at, winfried.maerz@luric-online.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Landesärztekammer Rheinland-Pfalz [LURIC, #837.255.97(1394)] and of Vorarlberg (VIVIT, EK-2-2013/0008). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GD, MK, BY, and AL designed and performed research, made statistical analyses, and drafted the manuscript. BK, WM, and HD designed research and corrected the manuscript. JS drafted the manuscript. HS measured serum uromodulin and corrected the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

LURIC was supported by the 7th Framework Program RiskyCAD (grant agreement number 305739) of the European Union and the H2020 Program TO_AITION (grant agreement number 848146) of the European Union. The work of WM and MK was supported as part of the Competence Cluster of Nutrition and Cardiovascular Health (nutriCARD) which was funded by the German Federal Ministry of Education and Research. The work of GD was supported by the European Union's Horizon 2020 research and innovation programme under the ERA-Net Cofund action N° 727565 (OCTOPUS project) and the German Ministry of Education and Research (grant number 01EA1801A). The funding sources were not involved in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

ACKNOWLEDGMENTS

We thank the LURIC study team who were either temporarily or permanently involved in patient recruitment as well as sample and data handling, in addition to the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg, Ulm, and Heidelberg, Germany. We are also grateful to the Vorarlberger Landesregierung (Bregenz, Austria) for continuously supporting the VIVIT research institute. We also thank Viktor Herbst, Matthias Block and Dr. Wolfgang Schlumberger, Institute of Experimental Immunology, Euroimmun AG Lübeck, for providing us with ELISA test kits, and Prof. Seymour Rosen, Beth Israel Deaconess MC, Harvard Medical School, Boston, and Prof. Olivier Devuyst, University Zürich, for valuable discussions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.723546/full#supplementary-material

REFERENCES

1. Kottgen A, Glazer NL, Dehghan A, Hwang SJ, Katz R, Li M, et al. Multiple loci associated with indices of renal function and chronic kidney disease. *Nat Genet.* (2009) 41:712–7. doi: 10.1038/ng.377
2. Kottgen A, Hwang SJ, Larson MG, Van Eyk JE, Fu Q, Benjamin EJ, et al. Uromodulin levels associate with a common UMOD variant and risk for incident CKD. *J Am Soc Nephrol.* (2010) 21:337–44. doi: 10.1681/ASN.2009070725
3. Köttgen A, Pattaro C, Böger CA, Fuchsberger C, Olden M, Glazer NL, et al. New loci associated with kidney function and chronic kidney disease. *Nat Genet.* (2010) 42:376–84. doi: 10.1055/s-0030-1266452
4. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. *PLoS Genet.* (2010) 6:e1001177. doi: 10.1097/01.hjh.0000378902.13083.3a
5. Ahluwalia TS, Lindholm E, Groop L, Melander O. Uromodulin gene variant is associated with type 2 diabetic nephropathy. *J Hypertens.* (2011) 29:1731–4. doi: 10.1097/HJH.0b013e328349de25
6. Devuyst O, Pattaro C. The UMOD locus: insights into the pathogenesis and prognosis of kidney disease. *J Am Soc Nephrol.* (2018) 29:713–26. doi: 10.1681/ASN.2017070716
7. Srivastava R, Micanovic R, El-Achkar TM, Janga SC. An intricate network of conserved DNA upstream motifs and associated transcription factors regulate the expression of uromodulin gene. *J Urol.* (2014) 192:981–9. doi: 10.1016/j.juro.2014.02.095
8. Schaeffer C, Devuyst O, Rampoldi L. Uromodulin: roles in health and disease. *Annu Rev Physiol.* (2021) 83:477–501. doi: 10.1146/annurev-physiol-031620-092817
9. Micanovic R, Chitteti BR, Dagher PC, Srour EF, Khan S, Hato T, et al. Tamm-Horsfall protein regulates granulopoiesis and systemic neutrophil homeostasis. *J Am Soc Nephrol.* (2015) 26:2172–82. doi: 10.1681/ASN.2014070664
10. Micanovic R, LaFavers K, Garimella PS, Wu XR, El-Achkar TM. Uromodulin (Tamm-Horsfall protein): guardian of urinary and systemic homeostasis. *Nephrol Dial Transplant.* (2019) 35:33–43. doi: 10.1093/ndt/gfy394
11. Delgado GE, Kleber ME, Scharnagl H, Kramer BK, Marz W, Scherberich JE. Serum uromodulin and mortality risk in patients undergoing coronary angiography. *J Am Soc Nephrol.* (2017) 28:2201–10. doi: 10.1681/ASN.2016111162
12. Leiherer A, Muendlein A, Saely CH, Ebner J, Brandtner EM, Fraunberger P, et al. Serum uromodulin is a predictive biomarker for cardiovascular events and overall mortality in coronary patients. *Int J Cardiol.* (2017) 231:6–12. doi: 10.1016/j.ijcard.2016.12.183
13. Steubl D, Buzkova P, Garimella PS, Ix JH, Devarajan P, Bennett MR, et al. Association of serum uromodulin with mortality and cardiovascular disease in the elderly-the Cardiovascular Health Study. *Nephrol Dial Transplant.* (2019) 35:1399–405. doi: 10.1093/ndt/gfz008
14. Garimella PS, Lee AK, Ambrosius WT, Bhatt U, Cheung AK, Chonchol M, et al. Markers of kidney tubule function and risk of cardiovascular disease events and mortality in the SPRINT trial. *Eur Heart J.* (2019) 40:3486–93. doi: 10.1093/eurheartj/ehz392
15. Then C, Then HL, Lechner A, Thorand B, Meisinger C, Heier M, et al. Serum uromodulin and risk for cardiovascular morbidity and mortality in the community-based KORA F4 study. *Atherosclerosis.* (2020) 297:1–7. doi: 10.1016/j.atherosclerosis.2020.01.030
16. Winkelmann BR, Marz W, Boehm BO, Zotz R, Hager J, Hellstern P, et al. Rationale and design of the LURIC study–a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics.* (2001) 2:S1–73. doi: 10.1517/14622416.2.1.S1
17. Leiherer A, Muendlein A, Kinz E, Vonbank A, Rein P, Fraunberger P, et al. High plasma chemerin is associated with renal dysfunction and predictive for cardiovascular events - insights from phenotype and genotype characterization. *Vascul Pharmacol.* (2016) 77:60–8. doi: 10.1016/j.vph.2015.08.010
18. Rein P, Vonbank A, Saely CH, Beer S, Jankovic V, Boehnel C, et al. Relation of albuminuria to angiographically determined coronary arterial narrowing in patients with and without type 2 diabetes mellitus and stable or suspected coronary artery disease. *Am J Cardiol.* (2011) 107:1144–8. doi: 10.1016/j.amjcard.2010.12.011
19. R Core Team. *R Foundation for Statistical Computing*. Vienna (2021).
20. Pivin E, Ponte B, de Seigneux S, Ackermann D, Guessous I, Ehret G, et al. Uromodulin and nephron mass. *Clin J Am Soc Nephrol.* (2018) 13:1556–7. doi: 10.2215/CJN.03600318
21. Pruijm M, Ponte B, Ackermann D, Paccaud F, Guessous I, Ehret G, et al. Associations of urinary uromodulin with clinical characteristics and markers of tubular function in the general population. *Clin J Am Soc Nephrol.* (2016) 11:70–80. doi: 10.2215/CJN.04230415
22. Devuyst O, Olinger E, Rampoldi L. Uromodulin: from physiology to rare and complex kidney disorders. *Nat Rev Nephrol.* (2017) 13:525–44. doi: 10.1038/nrneph.2017.101
23. Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O. The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. *Kidney Int.* (2011) 80:338–47. doi: 10.1038/ki.2011.134
24. Zhou J, Chen Y, Liu Y, Shi S, Wang S, Li X, et al. Urinary uromodulin excretion predicts progression of chronic kidney disease resulting from IgA nephropathy. *PLoS ONE.* (2013) 8:e71023. doi: 10.1371/journal.pone.0071023
25. Zylka A, Dumnicka P, Kusnierz-Cabala B, Gala-Bladzinska A, Ceranowicz P, Kucharz J, et al. Markers of glomerular and tubular damage in the early stage of kidney disease in type 2 diabetic patients. *Mediators Inflamm.* (2018) 2018:7659243. doi: 10.1155/2018/7659243
26. Bullen AL, Katz R, Lee AK, Anderson CAM, Cheung AK, Garimella PS, et al. The SPRINT trial suggests that markers of tubule cell function in the urine associate with risk of subsequent acute kidney injury while injury markers elevate after the injury. *Kidney Int.* (2019) 96:470–9. doi: 10.1016/j.kint.2019.03.024
27. Enko D, Meinitzer A, Scherberich JE, März W, Herrmann M, Artinger K, et al. Individual uromodulin serum concentration is independent of glomerular filtration rate in healthy kidney donors. *Clin Chem Lab Med.* (2020) 59:563–70. doi: 10.1515/cclm-2020-0894
28. Algharably EAH, Bolbrinker J, Lezius S, Reibis R, Wegscheider K, Voller H, et al. Uromodulin associates with cardiorenal function in patients with hypertension and cardiovascular disease. *J Hypertens.* (2017) 35:2053–8. doi: 10.1097/HJH.0000000000001432
29. Mutig K, Kahl T, Saritas T, Godes M, Persson P, Bates J, et al. Activation of the bumetanide-sensitive Na+,K+,2Cl- cotransporter (NKCC2) is facilitated by Tamm-Horsfall protein in a chloride-sensitive manner. *J Biol Chem.* (2011) 286:30200–10. doi: 10.1074/jbc.M111.222968
30. Trudu M, Janas S, Lanzani C, Debaix H, Schaeffer C, Ikehata M, et al. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. *Nat Med.* (2013) 19:1655–60. doi: 10.1038/nm.3384
31. Welker P, Böhlick A, Mutig K, Salanova M, Kahl T, Schlüter H, et al. Renal Na+-K+-Cl- cotransporter activity and vasopressin-induced trafficking are lipid raft-dependent. *Am J Physiol Renal Physiol.* (2008) 295:F789–802. doi: 10.1152/ajprenal.90227.2008
32. Olinger E, Lake J, Sheehan S, Schiano G, Takata T, Tokonami N, et al. Hepsin-mediated processing of uromodulin is crucial for salt-sensitivity and thick ascending limb homeostasis. *Sci Rep.* (2019) 9:12287. doi: 10.1038/s41598-019-48300-3
33. Graham LA, Aman A, Campbell DD, Augley J, Graham D, McBride MW, et al. Salt stress in the renal tubules is linked to TAL-specific expression of uromodulin and an upregulation of heat shock genes. *Physiol Genom.* (2018) 50:964–72. doi: 10.1152/physiolgenomics.00057.2018
34. Nie M, Bal MS, Liu J, Yang Z, Rivera C, Wu XR, et al. Uromodulin regulates renal magnesium homeostasis through the ion channel transient receptor potential melastatin 6 (TRPM6). *J Biol Chem.* (2018) 293:16488–502. doi: 10.1074/jbc.RA118.003950
35. Tokonami N, Takata T, Beyeler J, Ehrbar I, Yoshifuji A, Christensen EI, et al. Uromodulin is expressed in the distal convoluted tubule, where it is critical for regulation of the sodium chloride cotransporter NCC. *Kidney Int.* (2018) 94:701–15. doi: 10.1016/j.kint.2018.04.021
36. Leiherer A, Muendlein A, Saely CH, Brandtner EM, Geiger K, Fraunberger P, et al. The value of uromodulin as a new serum marker to predict decline in renal function. *J Hypertens.* (2018) 36:110–8. doi: 10.1097/HJH.0000000000001527

37. Thornley C, Dawnay A, Cattell WR. Human Tamm-Horsfall glycoprotein: urinary and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay. *Clin Sci.* (1985) 68:529–35. doi: 10.1042/cs0680529
38. Scherberich JE, Wolf G, Albers C, Nowack A, Stuckhardt C, Schoeppe W. Glomerular and tubular membrane antigens reflecting cellular adaptation in human renal failure. *Kidney Int Suppl.* (1989) 27:S38–51.
39. Steubl D, Block M, Herbst V, Nockher WA, Schlumberger W, Satanovskij R, et al. Plasma uromodulin correlates with kidney function and identifies early stages in chronic kidney disease patients. *Medicine.* (2016) 95:e3011. doi: 10.1097/MD.0000000000003011
40. Afsar B, Ortiz A, Covic A, Solak Y, Goldsmith D, Kanbay M. Focus on renal congestion in heart failure. *Clin Kidney J.* (2016) 9:39–47. doi: 10.1093/ckj/sfv124
41. Blankstein R, Bakris GL. Renal hemodynamic changes in heart failure. *Heart Fail Clin.* (2008) 4:411–23. doi: 10.1016/j.hfc.2008.03.006
42. Alesutan I, Luong TTD, Schelski N, Masyout J, Hille S, Schneider MP, et al. Circulating uromodulin inhibits vascular calcification by interfering with pro-inflammatory cytokine signaling. *Cardiovasc Res.* (2020) 117:930–41. doi: 10.1093/cvr/cvaa081

Conflict of Interest: WM reports grants and personal fees from AMGEN, BASF, Sanofi, Siemens Diagnostics, Aegerion Pharmaceuticals, Astrazeneca, Danone Research, Numares, Pfizer, Hoffmann LaRoche: personal fees from MSD, Alexion; grants from Abbott Diagnostics, all outside the submitted work. WM and MK are employed with Synlab Holding Deutschland GmbH. JS declares a patent at the University Charite, Berlin pending, and no other conflict of interest. BK reports travel support a/o lecture fees a/o advisory board memberships from Astellas, Bayer, BMS, Boehringer Ingelheim, Chiesi, Hexal, Pfizer, Sanofi, Servier, Vifor all outside the submitted work. MK reports lecture fees from Bayer outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 28 September 2021
doi: 10.3389/fmed.2021.745006

Sleeping, Smoking, and Kidney Diseases: Evidence From the NHANES 2017–2018

*Chia-Chao Wu[1,2], Han-En Wang[1], Yi-Chun Liu[3], Cai-Mei Zheng[4,5,6], Pauling Chu[1], Kuo-Cheng Lu[7], Chi-Ming Chu[3,8,9,10,11] and Yu-Tien Chang[3]**

[1] Division of Nephrology, Department of Internal Medicine, National Defense Medical Center, Tri-Service General Hospital, Taipei, Taiwan, [2] National Defense Medical Center, Department and Graduate Institute of Microbiology and Immunology, Taipei, Taiwan, [3] School of Public Health, National Defense Medical Center, Taipei City, Taiwan, [4] Division of Nephrology, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan, [5] TMU Research Centre of Urology and Kidney, Taipei Medical University, Taipei, Taiwan, [6] Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [7] Division of Nephrology, Department of Medicine, School of Medicine, Fu-Jen Catholic Hospital, Fu-Jen Catholic University, New Taipei City, Taiwan, [8] Department of Surgery, National Defense Medical Center, Songshan Branch of Tri-Service General Hospital, Taipei City, Taiwan, [9] Division of Biostatistics and Informatics, Department of Epidemiology, National Defense Medical Center, School of Public Health, Taipei, Taiwan, [10] Department of Public Health, China Medical University, Taichung City, Taiwan, [11] Department of Public Health, Kaohsiung Medical University, Kaohsiung City, Taiwan

Study Objectives: Smoking and sleep are modifiable factors associated with the chronic kidney diseases. However, the interaction of smoking and sleep on the renal function are still unclear. Therefore, we aimed to evaluate the interactive impacts of smoking and sleep on the renal function.

Methods: Data were obtained from the National Health and Nutrition Examination Survey. The study population were categorized into nine subgroups by smoking (smoking every day, sometimes, and non-smokers recently) and sleep duration (short duration $\leq$ 6 h, normal duration 6–9 h, and longer duration $\geq$ 9 h on the weekdays).

Results: The study group with a short sleep duration had significantly higher serum cotinine and hydrocotinine levels compared with the other two sleep groups. After adjusting the demographic characteristics (age, race, body mass index, and marital status), sleep quality (snoring or breathing cessation), and comorbidities (diabetes mellitus, hypertension, high cholesterol, anemia, congestive heart failure, coronary heart disease, and stroke), non-smokers with short or long sleep duration had significant lower estimated glomerular filtration rate (eGFR) levels than the study group who smoked every day and slept $\leq$6 h. The effects of sleep duration on eGFR levels varied with smoking status. For the study group smoking every day, eGFR levels increased as sleep duration decreased, whereas for the study group smoking sometimes, eGFR levels increased as sleep duration increased. The U-shaped effects of eGFR levels were observed among non-smokers whose normal sleep duration was associated with better eGFR levels. Normal sleep duration was an important protective factor of the renal function for non-smokers than smokers.

Conclusions: The effects of sleep duration on eGFR levels varied with smoking status. Normal sleep duration was a protective factor and more crucial for non-smokers than for smokers.

Keywords: sleep, smoking, kidney function, NHANES, eGFR

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Dirceu Silva, Clinical Hospital of Porto Alegre, Brazil
Andrea Abreu, University of São Paulo, Brazil

****Correspondence:***
Yu-Tien Chang
greengarden720925@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *21 July 2021*
Accepted: *30 August 2021*
Published: *28 September 2021*

Citation:
Wu C-C, Wang H-E, Liu Y-C, Zheng C-M, Chu P, Lu K-C, Chu C-M and Chang Y-T (2021) Sleeping, Smoking, and Kidney Diseases: Evidence From the NHANES 2017–2018. Front. Med. 8:745006. doi: 10.3389/fmed.2021.745006

INTRODUCTION

Chronic kidney diseases (CKDs) represent a heavy burden on the healthcare system because of the increasing number of patients, high risk of progression to end-stage renal disease, and poor prognosis with respect to morbidity and mortality (1). Sleep and smoking are two main modifiable factors of CKDs (2). Sleep plays an important role in every aspect of physiology. Sleep reduction has become highly prevalent owing to access to artificial indoor lighting, smartphones, and daily living activities. A population-based study showed that 22.3% of men and 28.9% of women aged $\geq$ 16 years told their doctors that they had trouble sleeping (3). Short sleep and long sleep duration (4) as well as poor objective sleep quality have been shown to be associated with the lower estimated glomerular filtration rate (eGFR) and CKD development (5–7).

Smoking is a leading cause of preventable deaths worldwide (8), and increases the risk of developing CKDs (9). The association of longer smoking duration with a higher risk of progression of CKDs was evident particularly in patients with eGFR $<$ 45 ml/min/1.73 m^2 and proteinuria $\geq$ 1.0 g/g. By contrast, the risk of adverse kidney outcomes decreased with longer smoking-free periods among former smokers (10).

Smoking and sleep problems have been demonstrated to have a reciprocal relationship with each other (10, 11). A strong relationship between smoking and subsequent sleep problems was observed in adolescents; this relationship was independent of demographics, snoring, or sleep apnea (SA), body mass index (BMI), depressive symptoms, alcohol use, and soda consumption (12). However, to the best of our knowledge, no study has evaluated the interaction effect of smoking and sleep duration on kidney function. Therefore, we aimed to evaluate the interactive impacts of smoking and sleep on renal function using datasets from the National Health and Nutrition Examination Survey (NHANES).

METHODS

Data Source

The National Health and Nutrition Examination Survey (13) is a program of studies designed to assess the health and nutritional status of adults and children in the United States. All the participants provided informed written consent for the study, which was approved by the Ethics Review Board of the National Center for Health Statistics. We used the NHANES datasets from 2017 to 2018, including all the cases of demographic variables (DEMO_J), questionnaire data of smoking and cigarette use (SMQ_J), sleep disorders (SLQ_J), laboratory data of albumin and creatinine—urine (ALB_CR_J), cotinine and hydroxycotinine—serum (COT_J), standard biochemistry profile (BIOPRO_J), blood pressure and cholesterol (BPQ_J), diabetes (DIQ_J), and medical conditions (MCQ_J).

Chronic Kidney Disease Epidemiology Collaboration Equations for eGFR

The R package of "CKDEpi.creat" (14) and parameters of serum creatinine, sex, age, and ethnicity were used to calculate eGFR using the CKD-EPI equation. The CKD-EPI equation is expressed as a single equation as follows: GFR $= 141 \times \min(S_{Cr}/\kappa, 1)^{\alpha} \times \max(S_{Cr}/\kappa, 1)^{-1.209} \times 0.993^{\text{age}} \times 1.018$ [if female] $\times$ 1.159 [if black], where S_{Cr} is the standardized serum creatinine in mg/dl, κ is 0.7 for women and 0.9 for men, a is -0.329 for women and -0.411 for men, min indicates the minimum of S_{Cr}/κ or 1, and max indicates the maximum of S_{Cr}/κ or 1 (15). eGFR values are presented in ml/min/1.73 m^2.

Statistical Analysis

Statistical analysis was performed using R version 4.0.2 (16). The testing index distribution was skewed; hence, we used the R package "bestNormalize" (17) to normalize the data. The testing index of blood urea nitrogen (mmol/l) was normalized using the center scale transformation whereas that of uric acid (μmol/l) was normalized using square root transformation. The levels of serum creatinine (μmol/l), urine creatinine (μmol/l), albumin–creatinine ratio (mg/g), urine albumin (μg/ml), serum cotinine (ng/ml), and serum hydroxycotinine (ng/ml) were normalized using log transformation. The descriptive statistics of the testing index are presented as non-normalized figures for clinical use. Transformed figures were used in the multivariable linear regression models. We performed univariable and multivariable linear regression analyses to determine whether sleep and smoking are associated with renal function while controlling for demographic characteristics (age, gender, and marriage), body measurement, marital status, sleep quality, and comorbidities. Variables that were significant in the univariable models were included in the multivariable analyses. The study population was divided into nine subgroups based on sleep duration on weekdays ($\leq$6, 6–9, and $\geq$ 9 h) and smoking (smoking every day, smoking sometimes, and never smoking recently) for sensitivity analysis. Forest plots were used to present the difference in eGFR levels among the nine subgroups.

RESULTS

Risk Factors of Kidney Diseases

The significant risk factors of kidney diseases were as follows: (1) demographic characteristics: male, older age, non-Hispanic white race, higher BMI and widowed/divorced or separated status; (2) sleep quality: frequent snoring or breath cession; (3) smoking: higher serum cotinine and hydrocotinine levels, older age at the start of smoking cigarettes regularly, no smoking recently, smoking since waking for 6–30 min, higher number of smoking days, number of cigarettes in the past 30 days; (4) comorbidities: hypertension, high cholesterol levels, diabetes mellitus, failing kidneys, anemia, congestive heart failure, coronary heart disease, stroke, chronic obstructive pulmonary disease, and malignancy. Blood urea nitrogen, serum creatinine, uric acid, and the albumin–creatinine ratio were negatively associated with eGFR levels (**Table 1**).

Baseline Characteristics and the Impacts of Sleep Duration on the Renal Functions

The normal sleep duration group (nmSleep) was the youngest (33.2 $\pm$ 24.3 years) and had the lowest BMI on average (25.6 $\pm$

TABLE 1 | Univariable linear regression of the renal function.

	eGFR (ml/min/1.73 m^2)	
	B	***p*-values**
Demographic characteristics		
Female (ref: male)	2.64	***
Age (year)	−1.07	***
Race (ref: Mexican American)		
Other Hispanic	−6.86	***
Non-Hispanic white	−15.36	***
Non-Hispanic black	−4.24	**
Other Race—including multi-racial	−5.43	***
Weight (kg)	−0.21	***
Height (cm)	−0.20	***
BMI (kg/m^2)	−0.64	***
Marital status (ref: Married or living with partner)		
Never married	12.55	***
Widowed, divorced, or separated	−11.51	***
Renal function		
Blood urea nitrogen (mmol/L)	−7.76	***
Creatinine, refrigerated serum (umol/L)	−0.40	***
Uric acid (umol/L)	−0.10	***
Albumin creatinine ratio (mg/g)	−0.01	***
Metabolites of nicotine		
Serum cotinine (ng/mL)	−0.02	***
Serum hydrocotinine (ng/mL)	−0.05	***
Sleep quality		
Sleep duration on weekdays (h)	−0.11	0.60
How often do you snore? (ref: never)		
Rarely—1–2 nights a week	−2.51	*
Occasionally—3–4 nights a week	−7.91	***
Frequently—5 or more nights a week	−8.60	***
How often do you snort or stop breathing (ref: never)		
Rarely—1–2 nights a week	−2.66	*
Occasionally—3–4 nights a week	−6.78	***
Frequently—5 or more nights a week	−10.90	***
Smoking status		
Age started smoking cigarettes regularly	−0.23	**
Do you now smoke cigarettes? (ref: Every day)		
Some days	0.93	0.63
Not at all	−12.41	***
How soon after waking do you smoke (ref: Within 5 min)		
6 ∼ 30 min	−4.95	*
≥30 min	−1.81	0.40
# days smoked cigarettes during past 30 days	−0.42	***
Average # cigarettes/day during past 30 days	−0.21	*
Tried to quit smoking	0.78	0.60
Comorbidities		
High blood pressure	−21.40	***
High cholesterol	−17.85	***
Diabetes (ref: Yes)		
No	22.64	***

(Continued)

TABLE 1 | Continued

	eGFR (ml/min/1.73 m^2)	
	B	***p*-values**
Borderline	10.27	***
Failing kidneys	−37.57	***
Anemia	−7.14	***
Congestive heart failure	−28.80	***
Coronary heart disease	−23.38	***
Stroke	−21.78	***
Chronic obstructive pulmonary disease	−16.74	***
Malignancy	−20.38	***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Ref, reference group.*

8.14 kg/m^2). The primary marital status of nmSleep was married or living with partners (60.5%) compared with the other two sleep groups with less or more sleep duration (abbreviation: lessSleep and moreSleep). Except for malignancy, nmSleep had a lower prevalence of hypertension, high cholesterol, DM, failing kidneys, anemia, congestive heart failure, coronary heart disease, heart attack, stroke, and COPD than the other two sleep groups (**Table 2**).

Renal function was associated with sleep duration. nmSleep and moreSleep had higher eGFR levels. nmSleep had the lowest blood urea nitrogen, the lowest serum creatinine, and a lower uric acid among the three sleep groups. No difference was found in the albumin–creatinine ratio. Taken together, nmSleep had the best renal function. The U-shaped effects of the renal function levels were observed among the sleep duration groups (**Table 2**). Significantly higher cotinine (75.6 ± 152 ng/ml, $p < 0.001$) and hydrocotinine (30.9 ± 82 ng/ml, $p < 0.001$) levels were observed in lessSleep compared with the other two sleep groups (**Table 2**; **Figure 1A**).

Baseline Characteristics and the Impacts of Smoking on the Renal Functions

The non-smoker group (noSmoking) was predominantly male (63.5%), non-Hispanic White (45.7%), married or living with partners, and older (58.8 ± 17 years) and had higher BMI (30.8 ± 7.39 kg/m^2). The prevalence of hypertension, high cholesterol, DM, failing kidneys, anemia, congestive heart failure, coronary heart disease, and malignancy was higher in noSmoking than other smoking groups of smoking every day (edSmoking) or smoking sometimes (stSmoking). The COPD prevalence was the highest in edSmoking (**Table 3**).

The non-smoker group had worse indices of the renal function than the other two smoking groups, but no difference was found in albumin–creatinine ratio levels (**Table 3**). The cotinine and hydrocotinine levels were positively associated with smoking frequency (**Table 3**; **Figure 1B**). They were highly correlated with each other as well with the Pearson correlation r 0.85 ($p < 0.001$) (**Figure 1C**).

Sleep Quality and Smoking Characteristics of the Study Population

Study population with sleep duration <6 h tended to had worse sleep qualities of snoring, breath cessation, and having trouble in sleeping. This sleep subgroup was more likely to smoke every day, smoke at least 100 cigarettes in life, and smoke within 30 min after waking up (**Table 4**). Over half percent of nmSleep and moreSleep did not smoke recently. The average sleep duration of edSmoking, stSmoking, and noSmoking were 7.42 ± 1.91, 7.50 ± 1.77, and 7.66 ± 1.66 h, respectively. There was no significant difference in the sleep qualities among the three smoking subgroups (**Table 5**). The U-shaped effects of cotinine levels among the three sleep subgroups were only observed in the study population smoked sometimes that normal sleep duration group had lower cotinine levels (**Figure 1D**).

The Interactive Impacts of Sleep Duration and Smoking on the Renal Function

The baseline eGFR levels of the study population who smoked every day or were sometimes similar and higher than the non-smokers (**Figure 2A**). Although the interaction term of smoking and sleep duration was not significant in the multivariable linear regression analysis (data not shown), the patterns of eGFR levels in the nine subgroups varied. For the study population who smoked every day, the longer the sleep time, the lower the eGFR levels. For non-smokers, the reverse U-shaped effects of eGFR levels were observed. Non-smokers with normal sleep duration had higher eGFR levels as compared with the other two sleep groups. For the study population who smoked sometimes, the longer sleep duration, the higher were the eGFR levels (**Figure 2B**).

After the adjustment of the demographic characteristics (age, race, BMI, and marital status), sleep quality (snoring or breathing cessation), and comorbidities (diabetes, high blood pressure, high cholesterol, anemia, congestive heart failure, coronary heart disease, and stroke), only noSmoking-lessSleep and noSmoking-moreSleep had significantly lowered eGFR levels compared with edSmoking-lessSleep. The U-shaped effect of sleep duration on the renal function was

TABLE 2 | Baseline characteristics of study population grouped by sleep duration.

	Sleep duration on weekdays			
	≤6 h (*n* = 1,118)	**6–9 h (*n* = 5,652)**	**≥9 h (*n* = 1,499)**	***p*-values**
Demographic characteristics				
Female	512 (45.8%)	2,874 (50.8%)	841 (56.1%)	***
Age (year)	48.5 (17.8)	33.3 (24.3)	49.1 (22.0)	***
Race				***
Mexican American	136 (12.2%)	848 (15.0%)	223 (14.9%)	
Other Hispanic	105 (9.39%)	489 (8.65%)	140 (9.34%)	
Non-Hispanic white	314 (28.1%)	1,907 (33.7%)	538 (35.9%)	
Non-Hispanic black	367 (32.8%)	1,211 (21.4%)	335 (22.3%)	
Other Race—including multi-racial	196 (17.5%)	1,197 (21.2%)	263 (17.5%)	
Weight (kg)	85.2 (24.9)	65.3 (30.4)	79.3 (22.2)	***
Height (cm)	167 (10.3)	155 (22.0)	165 (9.90)	***
BMI (kg/m^2)	30.3 (8.10)	25.6 (8.14)	28.9 (7.29)	***
Marital status				**
Married or living with partner	602 (57.6%)	1,949 (60.5%)	701 (54.1%)	
Never married	199 (19.0%)	569 (17.7%)	238 (18.4%)	
Widowed, divorced, or separated	245 (23.4%)	704 (21.8%)	356 (27.5%)	
Comorbidities				
High blood pressure	424 (37.9%)	1,181 (33.4%)	532 (35.6%)	*
High cholesterol	356 (32.2%)	1,117 (31.7%)	495 (33.2%)	0.60
Diabetes				***
Yes	154 (13.8%)	487 (8.62%)	252 (16.8%)	
No	926 (82.9%)	5,050 (89.4%)	1,212 (80.9%)	
Borderline	37 (3.31%)	113 (2.00%)	34 (2.27%)	
Failing kidneys	50 (4.79%)	100 (3.10%)	73 (5.64%)	***
Anemia	54 (4.84%)	177 (3.13%)	89 (5.97%)	***
Congestive heart failure	55 (5.27%)	85 (2.64%)	61 (4.72%)	***
Coronary heart disease	60 (5.74%)	121 (3.76%)	84 (6.49%)	***
Stroke	57 (5.47%)	118 (3.67%)	98 (7.56%)	***
Chronic obstructive pulmonary disease	71 (6.81%)	135 (4.19%)	87 (6.72%)	***
Malignancy	91 (8.71%)	334 (10.4%)	163 (12.6%)	***
Renal function				
Blood Urea Nitrogen (mmol/L)	5.36 (2.28)	5.14 (2.04)	5.33 (2.26)	**
Creatinine, refrigerated serum (umol/L)	82.6 (53.5)	75.1 (33.3)	79.6 (42.1)	***
eGFR (ml/min/1.73 m^2)	95.8 (25.5)	103 (28.8)	95.0 (28.6)	***
Uric acid (umol/L)	330 (87.8)	320 (87.9)	319 (88.4)	**
Albumin creatinine ratio (mg/g)	44.0 (223)	38.1 (294)	59.9 (365)	0.06
Metabolites of nicotine				
Serum cotinine (ng/mL)	75.6 (152)	30.4 (95.9)	49.7 (122)	***
Seum hydrocotinine (ng/mL)	30.9 (82.0)	11.7 (41.6)	21.4 (58.8)	***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ using statistical methods of ANOVA and Chi-squared test.*

significantly observed in noSmoking that nmSleep had better eGFR than the other two sleep groups (**Figure 3**). Normal sleep duration was a predominant profactor of the renal function in noSmoking. For the smokers, sleep duration had no significant effect on eGFR. A controversial finding is that noSmoking–moreSleep or noSmoking–lessSleep had lower eGFR than edSmoking–lessSleep in both the univariable and multivariable models (**Figure 3**).

DISCUSSION

Accumulating the clinical evidence suggests that cigarette smoking has a negative effect on the renal function, kidney dimensions (18), and CKD development of different etiologies, including DM, and hypertension (19, 20). Cigarette smoking is one of the most important modifiable renal risk factors (21). Nicotine, a major tobacco alkaloid, associates smoking with renal

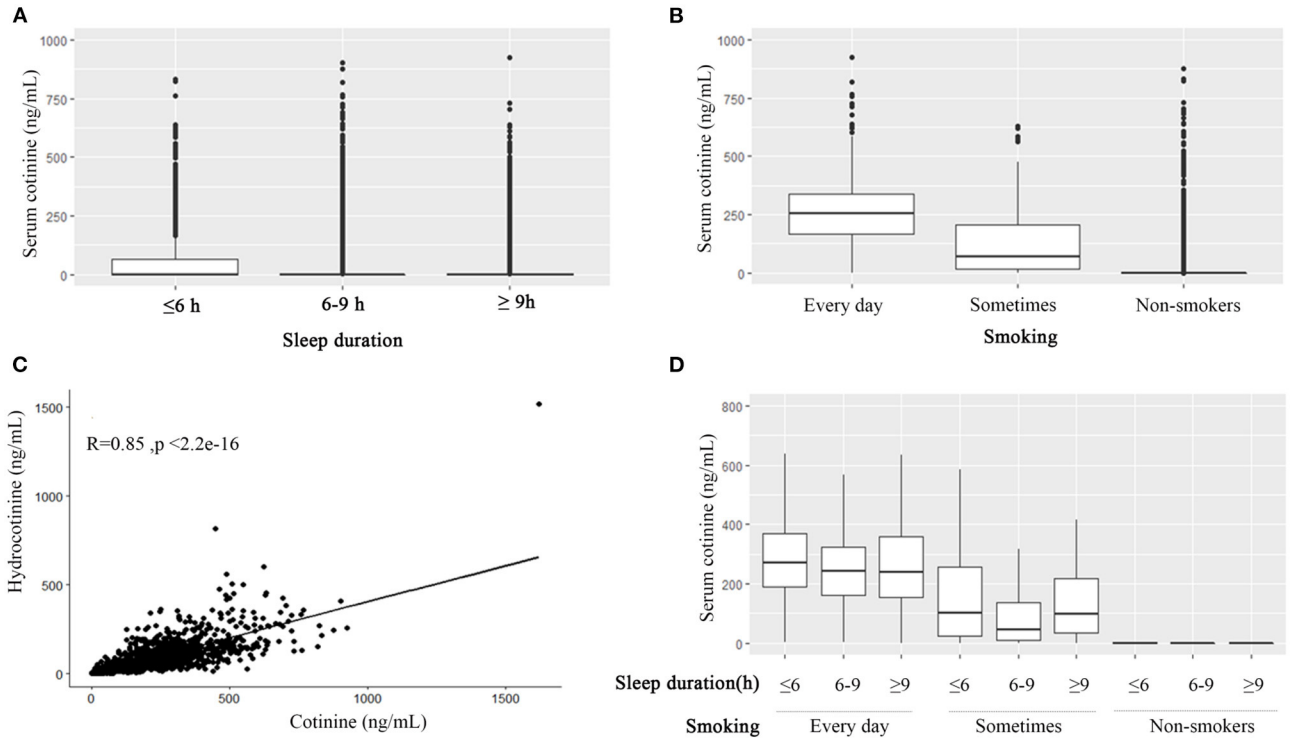


FIGURE 1 | The association among smoking, cotinine, hydrocotinine, and sleep duration. Boxplots of serum cotinine levels grouped by sleep duration (≤6, 6–9, and ≥9 h) **(A)**, grouped by smoking frequency **(B)**, and grouped by sleep duration and smoking frequency **(D)**. **(C)**The scatter plot of cotinine and hydrocotinine with the Pearson correlation r = 0.85.

dysfunction (22, 23). The risk of adverse kidney outcomes was incrementally higher as a smoking pack–years increased (24). Exposure to nicotine has been strongly shown to enhance renal oxidative stress (22) and kidney failure (25). Chronic exposure to nicotine accelerates the onset and progression of renal diseases in habitual cigarette smokers.

A major pathway of nicotine metabolism is C-oxidation, followed by cotinine; and the subsequent hydroxylation to trans-3′-hydroxycotinine. Moreover, 85–95% of the total nicotine uptake is eliminated as cotinine, hydroxycotinine, and glucuronides in the urine (26, 27). Cotinine has a longer plasma half-life than nicotine and showed a dose-dependent effect of smoking exposure (26, 28–30). This is in line with our finding; smoking frequency and serum cotinine and hydrocotinine levels were positively associated with each other. Therefore, we divided the smoking groups by the self-report of smoking frequency from the NHANES dataset.

The mechanisms of smoking-related renal damage are poorly understood, but the damage is likely caused by vascular and tubular effects (22). Smoking may sensitize the kidney to ischemic insults and perhaps facilitate the progression of acute kidney injury to chronic kidney injury (22). Nicotine increases the severity of renal injury in animal models leading to acute kidney injury, DM, acute nephritis, and subtotal nephrectomy (19). Nicotine stimulates the proliferation and hypertrophy of mesangial cells. Nicotine administration to sham rats increased total proteinuria but not albuminuria, indicating that nicotine directly affects tubular protein reabsorption (31). In humans, nicotine induces transitory increases in blood pressure accompanied by reductions in eGFR and effective renal plasma flow (19).

We found that the serum blood urea nitrogen and creatinine were higher and that eGFR levels were lower in the group of noSmoking than in the group of edSmoking and stSmoking after adjusting for age, race, BMI, marital status, and comorbidities (DM, hypertension, high cholesterol level, congestive heart failure, coronary heart disease, angina/angina pectoris, heart attack, stroke, emphysema, chronic bronchitis, and anemia). This finding is contradictory to the results of most studies (19–25).

In a study involving 28,409 individuals, smokers exhibited a slightly higher creatinine clearance rate than non-smokers at least in men after adjusting for hypertension (32). Moreover, the administration of nicotine to adolescent mice for 4 weeks incited higher oxidative stress and tubular injury than in adult kidney, but it did not modify creatinine levels (33). Does this higher creatinine clearance in smokers signify a better renal function? This increase may reflect a direct effect of smoking on tubular creatinine secretion or interfere with the estimation methods of creatinine. A 24-h urine collection would considerably eliminate any interference between smoking and estimation of creatinine

TABLE 3 | Baseline characteristics of study population grouped by smoking.

	Do you now smoking?			
	Every day (n = 805)	**Some days (n = 216)**	**Not at all (n = 1,338)**	***p*-values**
Demographic characteristics				
Female	336 (41.7%)	89 (41.2%)	488 (36.5%)	*
Age (year)	47.7 (16.0)	46.2 (16.1)	58.8 (17.0)	***
Race				***
Mexican American	57 (7.08%)	41 (19.0%)	170 (12.7%)	
Other Hispanic	41 (5.09%)	14 (6.48%)	118 (8.82%)	
Non-Hispanic white	368 (45.7%)	59 (27.3%)	611 (45.7%)	
Non-Hispanic black	233 (28.9%)	64 (29.6%)	256 (19.1%)	
Other Race—including multi-racial	106 (13.2%)	38 (17.6%)	183 (13.7%)	
Weight (kg)	82.7 (24.5)	86.6 (21.6)	87.4 (23.0)	***
Height (cm)	169 (9.31)	170 (9.65)	168 (9.43)	*
BMI (kg/m^2)	28.8 (8.11)	30.0 (6.86)	30.8 (7.39)	***
Marital status				***
Married or living with partner	397 (49.9%)	105 (50.0%)	809 (60.9%)	
Never married	184 (23.1%)	54 (25.7%)	135 (10.2%)	
Widowed, divorced, or separated	215 (27.0%)	51 (24.3%)	384 (28.9%)	
Comorbidities				
High blood pressure	292 (36.3%)	73 (33.8%)	669 (50.2%)	***
High cholesterol levels	215 (26.9%)	66 (31.3%)	635 (47.9%)	***
Diabetes				***
Yes	96 (11.9%)	27 (12.5%)	313 (23.4%)	
No	695 (86.3%)	183 (84.7%)	975 (72.9%)	
Borderline	14 (1.74%)	6 (2.78%)	49 (3.66%)	
Failing kidneys	19 (2.39%)	8 (3.81%)	91 (6.86%)	***
Anemia	31 (3.86%)	6 (2.79%)	70 (5.25%)	0.14
Congestive heart failure	27 (3.41%)	5 (2.38%)	101 (7.64%)	***
Coronary heart disease	29 (3.66%)	9 (4.29%)	123 (9.30%)	***
Stroke	59 (7.44%)	8 (3.81%)	93 (7.02%)	0.17
Chronic obstructive pulmonary disease	107 (13.5%)	13 (6.19%)	128 (9.66%)	**
Malignancy	69 (8.68%)	18 (8.57%)	214 (16.1%)	***
Renal function				
Blood Urea Nitrogen (mmol/L)	4.83 (1.83)	4.93 (1.84)	6.05 (2.60)	***
Creatinine, refrigerated serum (umol/L)	79.1 (23.1)	79.2 (21.9)	88.1 (45.0)	***
eGFR (ml/min/1.73 m^2)	96.5 (21.9)	97.5 (21.7)	84.1 (24.6)	***
Uric acid (umol/L)	321 (88.6)	325 (81.9)	347 (92.6)	***
Albumin creatinine ratio (mg/g)	37.1 (162)	34.7 (168)	58.4 (283)	0.11
Metabolites of nicotine				
Serum cotinine (ng/mL)	263 (139)	124 (140)	35.8 (118)	***
Serum hydrocotinine (ng/mL)	102 (76.9)	49.7 (57.6)	16.0 (61.6)	***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ using statistical methods of ANOVA and Chi-squared test.*

levels (32). The creatinine-based eGFR raises as the smoking amount increases, whereas the cystatin C-based eGFR decreases (34). This finding might indicate that creatinine-based eGFR, which was adopted in this study, may not be an ideal marker to estimate the relationship between smoking and renal function. Current smoking status cannot reflect the history of past exposure to cigarettes, and nicotine tends to be a short-term exposure marker of smoking. This is a limitation of this study. A high proportion of noSmoking individuals may have had a history of long past exposure to smoking cigarettes.

The finding of better renal function in smokers might have some biological plausibility. Lower doses of subacute nicotine administration can enhance renal function (35). Moreover, nicotine has a protective effect against neurotoxic insults and may

TABLE 4 | Sleep quality and smoking characteristics of study population grouped by sleep duration.

	Sleep duration on weekdays			
	≤6 h (n = 1,118)	6–9 h (n = 5,652)	≥9 h (n = 1,499)	p-values
Sleep quality				
Sleep duration (h)	5.23 (0.969)	7.54 (0.617)	9.74 (1.01)	***
How often do you snore				***
Never	284 (27.9%)	925 (28.2%)	479 (34.2%)	
Rarely−1–2 nights a week	226 (22.2%)	828 (25.2%)	316 (22.5%)	
Occasionally−3–4 nights a week	185 (18.2%)	648 (19.7%)	235 (16.8%)	
Frequently−5 or more nights a week	324 (31.8%)	881 (26.8%)	372 (26.5%)	
How often do you snore or have breath cessation				***
Never	778 (74.0%)	2,592 (77.6%)	1,104 (77.7%)	
Rarely−1–2 nights a week	133 (12.7%)	408 (12.2%)	158 (11.1%)	
Occasionally−3–4 nights a week	59 (5.61%)	200 (5.98%)	102 (7.18%)	
Frequently−5 or more nights a week	81 (7.71%)	142 (4.25%)	57 (4.01%)	
Having trouble sleeping	346 (30.9%)	858 (24.2%)	417 (27.9%)	***
Smoking status				
Smoked at least 100 cigarettes in life (yes/no)	489 (45.3%)	1,293 (38.4%)	577 (40.8%)	***
Age started smoking cigarettes regularly (years)	17.8 (6.06)	18.0 (6.53)	17.9 (6.33)	0.89
Do you now smoke cigarettes				**
Every day	202 (41.3%)	417 (32.3%)	186 (32.2%)	
Some days	50 (10.2%)	120 (9.28%)	46 (7.97%)	
Not at all	237 (48.5%)	756 (58.5%)	345 (59.8%)	
How soon after waking do you smoke				*
≤5 min	54 (27.7%)	85 (20.3%)	56 (31.3%)	
6 ∼ 30 min	66 (33.8%)	157 (37.5%)	48 (26.8%)	
≥30 min	75 (38.5%)	177 (42.2%)	75 (41.9%)	
# days smoked cigarettes during past 30 days	25.6 (8.65)	24.2 (10.0)	24.8 (9.19)	0.16
Average # cigarettes/day during past 30 days	11.2 (8.06)	11.2 (8.77)	10.3 (7.53)	0.42
Tried to quit smoking (yes/no)	126 (49.6%)	290 (53.0%)	125 (53.4%)	0.62

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ using statistical methods of ANOVA and Chi-squared test.*

be of potential therapeutic value in Parkinson's disease (36). In addition, cotinine reduced fear memory and anxiety after fear conditioning and improved working memory in a mouse model of Alzheimer's disease and in a monkey model of schizophrenia (37). Nicotine pretreatment reduced tubular damage (tubular cell apoptosis and proliferative response) due to an innate immune response in animal model experiments (38).

We found that the normal sleep duration of 6–9 h is associated with better eGFR and other renal function indices (blood urea nitrogen, creatinine, and uric acid) compared with the other two sleep duration groups; however, there was no difference in the albumin–creatinine ratio. In agreement with the previous studies, short and long sleep durations have been associated with adverse health outcomes in the general population (39, 40) and in patients with CKDs and diabetic kidney disease (DKD) (39, 41, 42). Overall, the cutoffs of normal sleep duration (6–8 or 6–9 h) may differ slightly, but the finding remained consistent (42). Physiological evidence indicated that sleep influences kidney function. The genetic risk score for short, but not long, sleep duration was significantly related with a higher risk of CKD stages 3–5 (6).

A poor sleep profile or quality is another important risk factor of increasing CKDs risk (42). SA is a condition that has serious health consequences, has an increased risk of death, and is common in patients with CKDs (43, 44) and DKD (45). Obstructive SA-related hypoxia causes several negative systemic effects, including oxidative stress (46, 47), inflammation, and sympathetic activation, all of which contribute to the progression of renal disease. In turn, CKD can result in the increased severity of SA by inducing uremic neuropathy and myopathy, altered chemosensitivity, and hypervolemia (43).

Sleeping behaviors and smoking have a reciprocal effect and a moderate correlation with genetics (48). Severe smoking status appears to have a causal effect on the circadian rhythm, and some evidence has shown that insomnia increases smoking heaviness and impedes cessation (48). Indeed, cigarette smoking has been shown to be associated with sleep disturbance *via* prolonged sleep-onset latency,

TABLE 5 | Sleep and smoking characteristics of study population grouped by smoking.

	Do you now smoking?			
	Every day (n = 805)	**Some days (n = 216)**	**Not at all (n = 1,338)**	***p*-values**
Sleep quality				
Sleep duration (h)	7.42 (1.91)	7.50 (1.77)	7.66 (1.66)	*
How often do you snore				0.14
Never	203 (27.5%)	53 (27.0%)	277 (22.8%)	
Rarely−1–2 nights a week	144 (19.5%)	49 (25.0%)	274 (22.6%)	
Occasionally−3–4 nights a week	136 (18.4%)	31 (15.8%)	242 (19.9%)	
Frequently−5 or more nights a week	255 (34.6%)	63 (32.1%)	422 (34.7%)	
How often do you snort or have breath cessation				0.59
Never	516 (70.3%)	159 (77.6%)	874 (71.0%)	
Rarely−1–2 nights a week	107 (14.6%)	23 (11.2%)	174 (14.1%)	
Occasionally−3–4 nights a week	61 (8.31%)	12 (5.85%)	95 (7.72%)	
Frequently−5 or more nights a week	50 (6.81%)	11 (5.37%)	88 (7.15%)	
Having trouble sleeping	296 (36.8%)	66 (30.6%)	464 (34.7%)	0.21
Smoking status				
Age started smoking cigarettes regularly (years)	17.9 (6.07)	18.9 (9.28)	17.8 (5.97)	0.07
How soon after waking do you smoke				
≤5 min	194 (24.9%)	–	–	
6 ∼ 30 min	270 (34.7%)	–	–	
≥30 min	315 (40.4%)	–	–	
# days smoked cigarettes during past 30 days	29.4 (2.99)	11.3 (7.15)	–	***
Average # cigarettes/day during past 30 days	12.8 (8.07)	4.13 (4.99)	–	***

*$p < 0.05$, ***$p < 0.001$ using statistical methods of ANOVA and Chi-squared test.

higher dopamine levels, and lower dopamine transporter levels in the cerebrospinal fluid of active smokers (49). The symptoms of cigarette smoking and nicotine dependence were associated with poor sleep quality in young adult smokers (50). Both oral nicotine administration and abstinence led to sleep disturbances in mice (51). In line with these findings, lessSleep individuals were shown to be more likely to smoke and had higher levels of cotinine and hydrocotinine in this study.

Many studies have discussed the individual association of sleep and smoking with the renal function in general, CKDs or DKD population. To the best of our knowledge, this is the first study to discuss the interaction effect of sleep duration and smoking on renal function. We observed that the non-smokers who had less or more sleep exhibited significantly lower eGFR levels compared with those who smoked every day and slept less. This association was independent of demographic characteristics (age, race, BMI, marital status), sleep quality of snoring or breathing cessation, and comorbidities (high blood pressure, high cholesterol, anemia, congestive heart failure, coronary heart disease, and stroke). This finding contradicts those of many studies that the risk of adverse kidney outcomes was incrementally higher as smoking pack–years increased (18, 19, 24). Even, we evaluated the eGFR of normal or abnormal serum cotinine groups, the average eGFR levels of the abnormal cotinine group were still slightly higher than the normal cotinine group. In noSmoking, the eGFR levels of the study population with normal levels of cotinine or hydrocotinine were lower than or equal to those with abnormal levels of cotinine.

The contradictory findings of smoking and nicotine on adverse kidney outcomes can be attributed to the following factors: (1) information on the nicotine exposure history of dose and length are not available (52), (2) creatinine-based eGFR may not be an ideal marker to estimate the relationship between smoking and the renal function (34), (3) other harmful ingredients from cigarettes have a greater effect on the renal function than nicotine (53), and (4) the non-smoking group possessed more risk factors (older age, male predominance, BMI, higher proportion of non-Hispanic white race, and higher prevalence of comorbidities). Despite our efforts to adjust for the potential confounders in the models, other residual confounders may still exist. During modeling, we found that after adjusting for age, the difference in eGFR levels between the smokers and non-smokers decreased. Older age played an important role in the development of adverse kidney function.

Among the non-smokers, sleep duration had a significant effect on eGFR. Either less or more sleep duration was harmful to the renal function, which was associated with a decline in eGFR. Normal sleep duration is an important profactor of the renal function in the non-smoking population. No

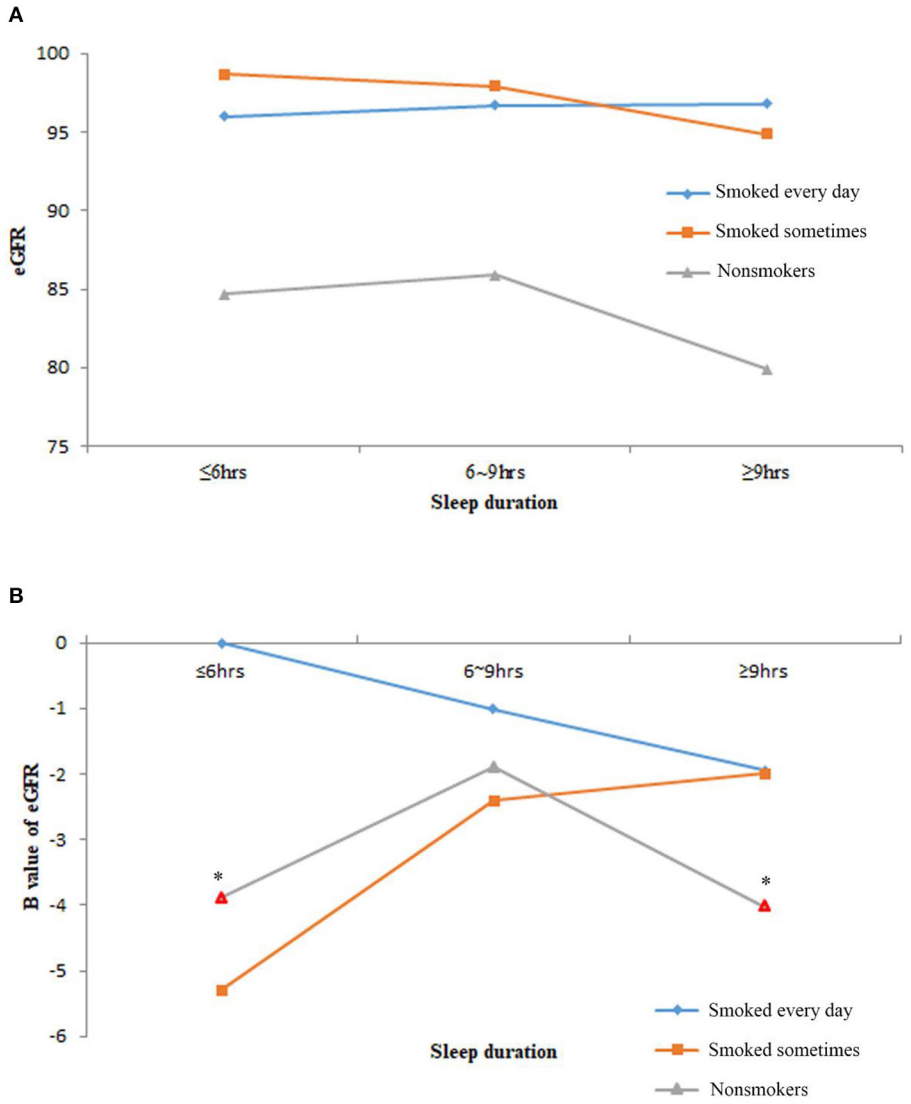


FIGURE 2 | The estimated glomerular filtration rate (eGFR) levels of the nine subgroups of study population by sleep duration and smoking frequency. **(A)** The average eGFR levels of nine subgroups. **(B)** The B value of eGFR in multivariable linear regression models after adjusting age, race, body mass index (BMI), marital status, frequency of snoring or breath cessation, diabetes, high blood pressure, high cholesterol, anemia, congestive heart failure, coronary heart disease and stroke. Marks in red border and asterisks denoted $p < 0.05$ in the multivariable linear regression models.

statistical significance of interaction effect was found between sleep duration and smoking status on eGFR in the multivariable linear regression models. However, the effects of sleep duration on eGFR levels varied with the smoking frequency. The eGFR levels of edSmoking increased as the sleep duration decreased, whereas the eGFR levels of stSmoking increased as the sleep duration increased. The U-shape effects of eGFR levels were observed among the non-smokers; the group with normal sleep duration had the highest eGFR levels. To the best of our knowledge, this is the first study to examine the interaction of sleep duration and smoking status on eGFR. However, because the NHANES datasets are cross-sectional, we were unable to understand the causal effects. Moreover, a reciprocal and prospective relationship exists between smoking and sleeping problems (12), and further research is required to unravel whether renal function has a reciprocal effect on sleep as well.

CONCLUSIONS

The effects of sleep duration on the renal function varied with smoking frequency. The non-smokers with short or long sleep duration exhibited significantly lower eGFR levels compared with those who smoked every day and

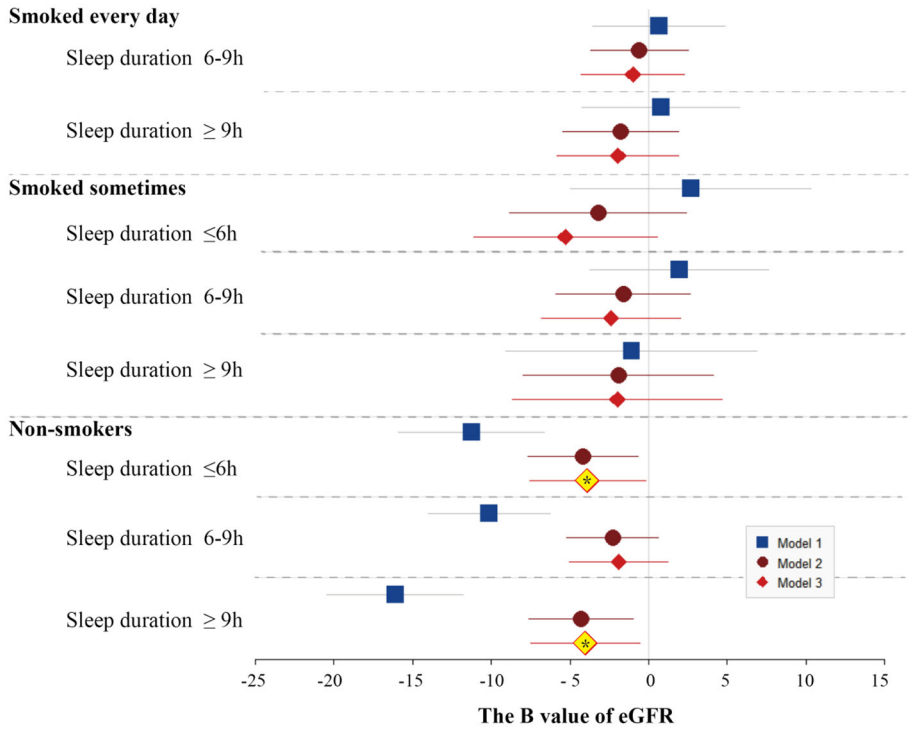


FIGURE 3 | Forest plots of B values of eGFR in the univariable and multivariable linear regression models among study population subgroups categorized by smoking and sleep duration. The subgroup of longer sleep duration and smoking every day was served as the reference group in the models. Model 1 was not adjusted. Model 2 was adjusted by age, race, BMI, and marital status. Model 3 was adjusted by age, race, BMI, marital status, frequency of snoring or breath cessation, diabetes, high blood pressure, high cholesterol, anemia, congestive heart failure, coronary heart disease, and stroke.

slept less after adjusting for demographic characteristics, sleep quality, and comorbidities. Normal sleep duration was a protective and more crucial factor for the non-smokers than for smokers. As this was a cross-sectional study, further longitudinal studies are required to confirm the causal effects of sleep and smoking on the renal function.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found at: National Health and Nutrition Examination Survey https://www.cdc.gov/nchs/nhanes/index.htm.

AUTHOR CONTRIBUTIONS

Y-CL and Y-TC: data curation. Y-TC: formal analysis, software, and writing the original draft. H-EW and C-CW: resources. C-CW: supervision. H-EW, C-CW, Y-CL, C-MZ, PC, K-CL, and C-MC: writing, reviewing, and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by research grants from the Tri-Service General Hospital (TSGH-C05-110033) and the Ministry of Science and Technology (MOST110-2314-B-016-014), Taiwan.

REFERENCES

1. Krishnamurthy S, Ks K, Dovgan E, Lustrek M, Gradisek Piletic B, Srinivasan K, et al. Machine learning prediction models for chronic kidney disease using national health insurance claim data in Taiwan. *Healthcare.* (2021) 9:13. doi: 10.3390/healthcare9050546
2. Neborak JM, Mokhlesi B. Short sleep, sleep apnoea-associated hypoxaemic burden and kidney function: more questions than answers. *Thorax.* (2021) 78:638–9. doi: 10.1136/thoraxjnl-2020-216618
3. Belal N, Mutter C, Khanna D. Prevalence of sleep disorders in a population aged 16 and older. *FASEB J.* (2020) 34(S1). doi: 10.1096/fasebj.2020.34.s1.09932
4. Jaussent I, Cristol JP, Stengel B, Ancelin ML, Dupuy AM, Besset A, et al. Impact of sleep disturbances on kidney function decline in the elderly. *Eur Respir J.* (2016) 47:860–8. doi: 10.1183/13993003.01147-2015
5. Calero K, Anderson WM. Can poor sleep cause kidney disease? Another step closer to the answer. *J Clin Sleep Med.* (2019) 15:371–2. doi: 10.5664/jcsm.7652
6. Park S, Lee S, Kim Y, Lee Y, Kang MW, Kim K, et al. Short or long sleep duration and CKD: a mendelian randomization study. *J Am Soc Nephrol.* (2020) 31:2937–47. doi: 10.1681/ASN.2020050666
7. Knutson KL, Lash J, Ricardo AC, Herdegen J, Thornton JD, Rahman M, et al. Habitual sleep and kidney function in chronic kidney disease: the Chronic Renal Insufficiency Cohort study. *J Sleep Res.* (2018) 27:281–9. doi: 10.1111/jsr.12573

8. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* (2012) 380:2224–60. doi: 10.1016/S0140-6736(12)61766-8
9. Choi HS, Han KD, Oh TR, Kim CS, Bae EH, Ma SK, et al. Smoking and risk of incident end-stage kidney disease in general population: a Nationwide Population-based Cohort Study from Korea. *Sci Rep.* (2019) 9:19511. doi: 10.1038/s41598-019-56113-7
10. Patten CA, Choi WS, Gillin JC, Pierce JP. Depressive symptoms and cigarette smoking predict development and persistence of sleep problems in US adolescents. *Pediatrics.* (2000) 106:E23. doi: 10.1542/peds.106.2.e23
11. Wong MM, Brower KJ, Zucker RA. Childhood sleep problems, early onset of substance use and behavioral problems in adolescence. *Sleep Med.* (2009) 10:787–96. doi: 10.1016/j.sleep.2008.06.015
12. Bellatorre A, Choi K, Lewin D, Haynie D, Simons-Morton B. Relationships between smoking and sleep problems in black and white adolescents. *Sleep.* (2017) 40:8. doi: 10.1093/sleep/zsw031
13. Centers for Disease Control Prevention (CDC), National Center for Health Statistics (NCHS), MD: U.S. Department of Health and Human Services CfDCa. *National Health and Nutrition Examination Survey Data.* (2021). Available online at: https://www.cdc.gov/nchs/nhanes/index.htm
14. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* (2012) 367:20–9. doi: 10.1056/NEJMoa1114248
15. Stevens LA, Levey AS. Measured GFR as a confirmatory test for estimated GFR. *J Am Soc Nephrol.* (2009) 20:2305–13. doi: 10.1681/ASN.2009020171
16. R Core Team. *R: A Language and Environment for Statistical Computing* (2020).
17. Peterson RA, Cavanaugh JE. Ordered quantile normalization: a semiparametric transformation built for the cross-validation era. *J Appl Stat.* (2019) 47:1–16. doi: 10.1080/02664763.2019.1630372
18. Farhat A, Jones IA, Saadat S, Dornhofer K, Kong C, Nguyen T, et al. The association of smoking with ultrasound-measured kidney dimensions. *Clin Nephrol.* (2020) 93:9–16. doi: 10.5414/CN109854
19. Jain G, Jaimes EA. Nicotine signaling and progression of chronic kidney disease in smokers. *Biochem Pharmacol.* (2013) 86:1215–23. doi: 10.1016/j.bcp.2013.07.014
20. Leonberg-Yoo AK, Rudnick MR. Tobacco use: a chronic kidney disease accelerant. *Am J Nephrol.* (2017) 46:257–9. doi: 10.1159/000481209
21. Orth SR. Cigarette smoking: an important renal risk factor - far beyond carcinogenesis. *Tob Induc Dis.* (2002) 1:137–55. doi: 10.1186/1617-9625-1-2-137
22. Arany I, Grifoni S, Clark JS, Csongradi E, Maric C, Juncos LA. Chronic nicotine exposure exacerbates acute renal ischemic injury. *Am J Physiol Renal Physiol.* (2011) 301:F125–33. doi: 10.1152/ajprenal.00041.2011
23. Sanner T, Grimsrud TK. Nicotine: carcinogenicity and effects on response to cancer treatment - a review. *Front Oncol.* (2015) 5:196. doi: 10.3389/fonc.2015.00196
24. Lee S, Kang S, Joo YS, Lee C, Nam KH, Yun HR, et al. Smoking, smoking cessation, and progression of chronic kidney disease: results from KNOW-CKD study. *Nicotine Tob Res.* (2021) 23:92–8. doi: 10.1093/ntr/ntaa071
25. Ramalingam A, Santhanathas T, Shaukat Ali S, Zainalabidin S. Resveratrol supplementation protects against nicotine-induced kidney injury. *Int J Environ Res Public Health.* (2019) 16:13. doi: 10.3390/ijerph16224445
26. Yamazaki H, Horiuchi K, Takano R, Nagano T, Shimizu M, Kitajima M, et al. Human blood concentrations of cotinine, a biomonitoring marker for tobacco smoke, extrapolated from nicotine metabolism in rats and humans and physiologically based pharmacokinetic modeling. *Int J Environ Res Public Health.* (2010) 7:3406–21. doi: 10.3390/ijerph7093406
27. Nakajima M, Yokoi T. Interindividual variability in nicotine metabolism: C-oxidation and glucuronidation. *Drug Metab Pharmacokinet.* (2005) 20:227–35. doi: 10.2133/dmpk.20.227
28. Kunutsor SK, Spee JM, Kieneker LM, Gansevoort RT, Dullaart RPF, Voerman AJ, et al. Self-reported smoking, urine cotinine, and risk of cardiovascular disease: findings from the PREVEND (Prevention of Renal and Vascular End-Stage Disease) Prospective Cohort Study. *J Am Heart Assoc.* (2018) 7:15. doi: 10.1161/JAHA.118.008726
29. Hellemons ME, Sanders JS, Seelen MA, Gans RO, Muller Kobold AC, van Son WJ, et al. Assessment of cotinine reveals a dose-dependent effect of smoking exposure on long-term outcomes after renal transplantation. *Transplantation.* (2015) 99:1926–32. doi: 10.1097/TP.0000000000000636
30. Torres S, Merino C, Paton B, Correig X, Ramirez N. Biomarkers of exposure to secondhand and thirdhand tobacco smoke: recent advances and future perspectives. *Int J Environ Res Public Health.* (2018) 15:25. doi: 10.3390/ijerph15122693
31. Rezonzew G, Chumley P, Feng W, Hua P, Siegal GP, Jaimes EA. Nicotine exposure and the progression of chronic kidney disease: role of the alpha7-nicotinic acetylcholine receptor. *Am J Physiol Renal Physiol.* (2012) 303:F304–12. doi: 10.1152/ajprenal.00661.2011
32. Halimi JM, Giraudeau B, Vol S, Caces E, Nivet H, Lebranchu Y, et al. Effects of current smoking and smoking discontinuation on renal function and proteinuria in the general population. *Kidney Int.* (2000) 58:1285–92. doi: 10.1046/j.1523-1755.2000.00284.x
33. Arany I, Hall S, Dixit M. Age-dependent sensitivity of the mouse kidney to chronic nicotine exposure. *Pediatr Res.* (2017) 82:822–8. doi: 10.1038/pr.2017.153
34. Ohkuma T, Nakamura U, Iwase M, Ide H, Fujii H, Jodai T, et al. Effects of smoking and its cessation on creatinine- and cystatin C-based estimated glomerular filtration rates and albuminuria in male patients with type 2 diabetes mellitus: the Fukuoka Diabetes Registry. *Hypertens Res.* (2016) 39:744–51. doi: 10.1038/hr.2016.51
35. Akomolafe OR, Imafidon CE, Olukiran OS, Oladele AA, Akanji BO. Sub-acute administration of lower doses of nicotine caused sex-dependent improvement of renal function in Wistar rats. *Toxicol Rep.* (2017) 4:535–42. doi: 10.1016/j.toxrep.2017.10.001
36. Riveles K, Huang LZ, Quik M. Cigarette smoke, nicotine and cotinine protect against 6-hydroxydopamine-induced toxicity in SH-SY5Y cells. *Neurotoxicology.* (2008) 29:421–7. doi: 10.1016/j.neuro.2008.02.001
37. Moran VE. Cotinine: beyond that expected, more than a biomarker of tobacco consumption. *Front Pharmacol.* (2012) 3:173. doi: 10.3389/fphar.2012.00173
38. Sadis C, Teske G, Stokman G, Kubjak C, Claessen N, Moore F, et al. Nicotine protects kidney from renal ischemia/reperfusion injury through the cholinergic anti-inflammatory pathway. *PloS one.* (2007) 2:e469. doi: 10.1371/journal.pone.0000469
39. Lee HJ, Kwak N, Kim YC, Choi SM, Lee J, Park YS, et al. Impact of sleep duration on mortality and quality of life in chronic kidney disease: results from the 2007-2015 KNHANES. *Am J Nephrol.* (2021) 52:1–8. doi: 10.1159/000516096
40. Hao Q, Xie M, Zhu L, Dou Y, Dai M, Wu Y, et al. Association of sleep duration with chronic kidney disease and proteinuria in adults: a systematic review and dose-response meta-analysis. *Int Urol Nephrol.* (2020) 52:1305–20. doi: 10.1007/s11255-020-02488-w
41. Tan NYQ, Chan J, Cheng CY, Wong TY, Sabanayagam C. Sleep duration and diabetic kidney disease. *Front Endocrinol.* (2018) 9:808. doi: 10.3389/fendo.2018.00808
42. Bo Y, Yeoh EK, Guo C, Zhang Z, Tam T, Chan TC, et al. Sleep and the risk of chronic kidney disease: a cohort study. *J Clin Sleep Med.* (2019) 15:393–400. doi: 10.5664/jcsm.7660
43. Hui L, Benca R. The bidirectional relationship between obstructive sleep apnea and chronic kidney disease. *J Stroke Cerebrovasc Dis.* (2021) 30:105652. doi: 10.1016/j.jstrokecerebrovasdis.2021.105652
44. Wang Y, Meagher RB, Ambati S, Cheng H, Ma P, Phillips BG. Patients with obstructive sleep apnea have altered levels of four cytokines associated with cardiovascular and kidney disease, but near normal levels with airways therapy. *Nat Sci Sleep.* (2021) 13:457–66. doi: 10.2147/NSS.S282869
45. Meng L, Ding Y, Li J, Li X, Yan T, Yang M, et al. Impact of inflammatory markers on the relationship between sleep quality and diabetic kidney disease. *Sleep Breath.* (2021). doi: 10.1007/s11325-021-02380-6. [Epub ahead of print].
46. Jackson CL, Umesi C, Gaston SA, Azarbarzin A, Lunyera J, McGrath JA, et al. Multiple, objectively measured sleep dimensions including hypoxic burden and chronic kidney disease: findings from the Multi-Ethnic Study of Atherosclerosis. *Thorax.* (2020) 76:704–13. doi: 10.1136/thoraxjnl-2020-214713
47. Jhamb M, Ran X, Abdalla H, Roumelioti ME, Hou S, Davis H, et al. Association of sleep apnea with mortality in patients with advanced kidney

disease. *Clin J Am Soc Nephrol.* (2020) 15:182–90. doi: 10.2215/CJN.07880719
48. Gibson M, Munafo MR, Taylor AE, Treur JL. Evidence for genetic correlations and bidirectional, causal effects between smoking and sleep behaviors. *Nicotine Tob Res.* (2019) 21:731–8. doi: 10.1093/ntr/nty230
49. Li H, Liu Y, Xing L, Yang X, Xu J, Ren Q, et al. Association of cigarette smoking with sleep disturbance and neurotransmitters in cerebrospinal fluid. *Nat Sci Sleep.* (2020) 12:801–8. doi: 10.2147/NSS.S272883
50. Dugas EN, Sylvestre MP, O'Loughlin EK, Brunet J, Kakinami L, Constantin E, et al. Nicotine dependence and sleep quality in young adults. *Addict Behav.* (2017) 65:154–60. doi: 10.1016/j.addbeh.2016.10.020
51. Mathews HL, Stitzel JA. The effects of oral nicotine administration and abstinence on sleep in male C57BL/6J mice. *Psychopharmacology.* (2019) 236:1335–47. doi: 10.1007/s00213-018-5139-6
52. Speeckaert MM, Delanghe JR, Vanholder RC. Chronic nicotine exposure and acute kidney injury: new concepts and experimental evidence. *Nephrol Dial Transplant.* (2013) 28:1329–31. doi: 10.1093/ndt/gft019
53. Cooper RG. Effect of tobacco smoking on renal function. *Indian J Med Res.* (2006) 124:261–8.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

REVIEW
published: 12 November 2021
doi: 10.3389/fmed.2021.704409

Obesity Facts and Their Influence on Renal Function Across the Life Span

*Vera H. Koch**

Pediatric Nephrology Unit, Department of Pediatrics, Instituto da Criança e do Adolescente do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Obesity is a chronic disease, with a rapidly increasing prevalence worldwide. Body mass index (BMI) provides the most useful population-level measure of overweight and obesity. For adults, overweight is defined as a BMI (Kg/m^2) $\geq$ 25, and obesity as a BMI $\geq$ 30, for non-Asians and $\geq$ 27.5 for Asians. Abdominal obesity can be defined as a waist circumference equal to or higher than 102 cm for men and $\geq$88 cm for women. The definition of children and adolescents BMI changes with age and sex. Obesity may be exogenous or endogenous obesity, the latter is multifactorial and predominantly manifested during childhood. Presently, overweight and obesity are linked to more deaths worldwide than underweight. The total kidney glomerular filtration rate (GFR) is determined by the sum of nephrons and the GFR within each nephron or single nephron GFR. In clinical practice, GFR is more frequently calculated by GFR estimating equations based upon the plasma levels of creatinine, cystatin C, or both. The measured value of plasma creatinine is strongly influenced by non-GFR factors, by its tubular and gastrointestinal secretion, and by the problems associated with the lack of standardization of creatinine's laboratory assay discrediting it as an ideal GFR biomarker. Unlike creatinine, cystatin C plasma levels are mainly determined by GFR. Obesity may affect the kidney, *via* development of systemic arterial hypertension and/or diabetes mellitus, or directly, by ectopic accumulation of adipose tissue in the kidney. As obesity is a clinical condition associated with altered body composition, creatinine may not be the ideal biomarker for GFR measurement in obese individuals.

Keywords: obesity, glomerular filtration rate, creatinine, cystatin C, pediatric, adult

OPEN ACCESS

Edited by:
Ana Cusumano, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Argentina

Reviewed by:
Mark Dominik Alscher, Robert Bosch Hospital, Germany
Pierluigi Marzuillo, Second University of Naples, Italy

***Correspondence:**
Vera H. Koch
vera.koch23@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *02 May 2021*
Accepted: *08 September 2021*
Published: *12 November 2021*

Citation:
Koch VH (2021) Obesity Facts and Their Influence on Renal Function Across the Life Span. Front. Med. 8:704409. doi: 10.3389/fmed.2021.704409

INTRODUCTION

The global prevalence of obesity has nearly tripled between 1975 and 2016. Approximately 13% of the world's adult population (11% of men and 15% of women) could be classified as obese in 2016. In the same period, the prevalence of overweight and obesity among children and adolescents, aged 5–19, of both sexes, has risen from 4% in 1975 to over 18% in 2016[1].

Obesity is a chronic disease, which results from long-term positive energy balance with development of excessive body fat mass. It leads to structural and functional abnormalities potentially associated with an elevated to premature mortality risk (1). Body mass index (BMI) provides the most useful, although rough, population-level measure of overweight and obesity but as BMI cannot differentiate between lean and fat body mass, it may not correspond to the same degree of fatness in different individuals[1].

[1]https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed Aug 22, 2021).

THE DEFINITION OF OVERWEIGHT AND OBESITY ACROSS THE LIFESPAN

For adults, overweight is defined as a BMI ≥ 25 Kg/m^2, and obesity as a BMI ≥ 30 Kg/m^2, for non-Asians and ≥27.5 Kg/m^2 for Asians and abdominal obesity can be defined as a waist circumference ≥102 cm for men and ≥88 cm for women (2)[1].

In children and adolescents, growth, and maturation lead to changes of BMI with age and sex. There are at least three different definition proposals for child and adolescent overweight and obesity, which have been issued by the WHO, the Center for Disease Control and Prevention CDC) and by the International Obesity Task Force (IOTF). The WHO criteria recommend utilization of BMI for adolescents and weight- for- height/ length *Z*-score for children (3). The Center for Disease Control and Prevention (CDC) has derived sets of percentiles of age and sex-specific BMI for children and adolescents aged 2–20 years, in the United States (4). The International Obesity Task Force (IOTF) obtained data on body mass index (weight/height) from six large cross-sectional surveys on growth from Brazil, Great Britain, Hong Kong, the Netherlands, Singapore, and the US, including 97,876 males and 94,851 females from birth to 25 years of age. For each of the surveys, centile curves were drawn passing through the established cut-off points of 25 and 30 kg/m^2 for adult weight and obesity, at age 18 years. The resulting curves were averaged to provide age- and sex- specific cut-off points from 2 to 18 years (5). Despite a consensus between the two most frequently used criteria, CDC and IOTF, their discrepancies about age, gender, and country are noticeable and render the estimated prevalence rates of overweight and obesity non-comparable worldwide (6).

For children >2 years of age, using the CDC criteria, overweight may be defined as BMI ≥85th percentile but <95th percentile for age and sex, obesity as BMI ≥95th percentile, and extreme obesity if the BMI is ≥120% of the 95th percentile or ≥35 kg/m^2 (7). For children <2 years of age, obesity may be defined as the sex-specific weight for recumbent length ≥97.7th percentile on the World Health Organization (WHO) charts (7). A new classification system recognizes BMI ≥95th percentile as class I obesity, BMI ≥ 120% of the 95th percentile as class II obesity, and BMI ≥ 140% of the 95th percentile as class III obesity. Class II and III obesity are strongly associated with greater cardiovascular and metabolic risk (8).

Table 1 presents a summary of the cut -off values used to define overweight and obesity in the pediatric age range and in adulthood.

Waist circumference (WC), waist-to-hip ratio and waist-to-height ratio (WHtR) reference percentiles are used to define abdominal obesity in children and adolescents, these indices may vary according to ethnicity (10–13). Observations from pediatric and adult studies suggest WHtR and WC to be independent and more significant predictors of cardiometabolic outcomes than BMI, Mean threshold values for WHtR, covering all cardiometabolic outcomes, from studies in fourteen different countries and including Caucasian, Asian and Central American subjects, were 0.50 for men and 0.50 for women. The AUROC analyses indicate that WHtR may be a more useful global clinical screening tool than WC, with a weighted mean threshold value of 0.5, or in simpler terms, for public health purposes, the "waist circumference should be kept to less than half the individual's height" (14).

TABLE 1 | Cut-off values used to define overweight and obesity in childhood and adulthood.

Overweight	Obesity
CHILDREN AND ADOLESCENTS	
CDC Children 2–20 years of age	
BMI ≥85th percentile but <95th percentile for age and sex	BMI ≥95th percentile
WHO children <5 years of age	
BMI or Weight for length/ height > +2SD WHO Child Growth Standards median	Obese: BMI or Weight for length/ height > +3SD WHO Child Growth Standards median
WHO 5–19 years of age	
BMI > +1SD WHO Growth Reference median;	BMI > +2 SD WHO Growth Reference median;
IOTH 2–18 years of age	
Age- and sex- specific cut-off points to correspond to BMI cut-offs of 25 kg/m^2 at 18 years of age	Age- and sex- specific cut-off points to correspond to BMI cut-offs of 30 kg/m^2 at 18 years of age
ADULTS	
BMI ≥ 25 Kg/m^2	BMI ≥30 Kg/m^2 for non- Asians and ≥ 27.5 Kg/m^2 for Asians

Source: Modified from references (1, 2, 7, 9)[1].
CDC, Centers for Disease Control and Prevention; IOTF, International Obesity Task Force; BMI, Body Mass Index; WHO, World Health Organization.

OBESITY AS A MULTIFACTORIAL ENTITY

The Etiology of Obesity May Be Endogenous or Exogenous

The development of exogenous or acquired obesity requires chronic overconsumption, leading to storage of excess caloric intake as lipid in fat cells, without behavioral or metabolic compensation (15). In healthy individuals, 70–95% of insulin-mediated glucose disposal is undertaken by skeletal muscle-cells. The functional compromise of skeletal muscle-cell metabolic-flux and concomitant insulin sensitivity, which are the main determinants of metabolic control, lead to increased availability of glucose or other serum energy substrates for adipogenesis. Of note, the major modifiable determinant of skeletal muscle cell insulin sensitivity is physical activity, extremely low levels of physical activity decreases both insulin sensitivity and metabolic control (15).

Over the past few generations, physical activity and fitness levels declined in both children and adults (16). When an individual's physical activity falls below the "metabolic tipping point," defined as the minimum amount of physical activity and associated metabolic flux, necessary to avoid positive energy-balance, acquired obesity develops (15).

Various monogenic, genetic syndromic, and endocrine conditions may lead to endogenous obesity, which is predominantly manifested during childhood. The assessment

of childhood endogenous obesity should be based on a very thorough history and physical examination directed at etiological elucidation and diagnosis of the associated metabolic, cardiovascular, respiratory, gastrointestinal, orthopedic, and psychological complications (9). Endocrine causes, although rare (2–3% of all referrals for pediatric obesity evaluation) should be carefully evaluated so that specific treatment can be provided (16). Children born small or large- for- gestational age, infants of diabetic mothers and those with rapid or excessive catch-up growth in the first few years of life are frequently conditioned by metabolic programming, which exacerbates the problem of obesity associated with lifestyle and dietary factors (17). **Table 2** lists the main etiologies associated with endogenous and exogenous obesity.

Presently, overweight and obesity are linked to more deaths worldwide than underweight[1]. An analytical study carried out on 10,625,411 subjects in Asia, Australia and New Zealand, Europe, and North America, from 239 prospective studies, evaluated mortality risk, paired by sex and age, compared to BMI 22.5–25.0. The study confirmed the associations of both overweight and obesity, with increased mortality from any cause, within the four continents (18).

Prediabetes /type 2 diabetes mellitus; dyslipidemia; prehypertension / hypertension; sleep apnea; non-alcoholic fatty liver disease; proteinuria and focal segmental glomerulosclerosis; early subclinical atherosclerosis; cardiovascular disease (CVD), hyperandrogenemia/ polycystic ovary syndrome, and orthopedic problems are among the multiple clinical comorbidities associated with pediatric overweight and obesity. Obesity severity is associated with higher cardiometabolic risk factors and premature mortality in adulthood (7). Of note, the risks of CVD outcomes among obese children and adolescents who became non-obese by adulthood are similar to those who were never obese (19).

TABLE 2 | Main etiologies associated with endogenous and exogenous obesity.

EXOGENOUS OBESITY	
Chronic overconsumption	Storage of excess caloric intake as lipid in fat cells, without behavioral or metabolic compensation
Medications	Glucocorticoids, tricyclic antidepressants, risperidone
Adverse perinatal metabolic programming	Children born small or large- for- gestational age
ENDOGENOUS OBESITY	
Monogenic diseases	
Recessive defects in genes	
Leptin (LEP)	Extreme hyperphagia, frequent infections, hypogonadotropic hypogonadism, hypothyroidism
Leptin receptor (LEPR)	Same as LEP
Pro-opiomelanocortin (POMC)	Hyperphagia, cholestatic jaundice or adrenal crisis due to ACTH deficiency, pale skin, and red hair in Caucasians
Proprotein convertase subtilisin/kexin type 1 (PCSK1)	Small bowel enteropathy, hypoglycemia, hypothyroidism, ACTH deficiency, diabetes insipidus
Dominant defects in genes	
Melanocortin 4 receptor (MC4R),	Hyperphagia, accelerated linear growth, disproportionate hyperinsulinemia, low/normal blood pressure
Src homology 2 B adapter protein 1 (SH2B1)	Hyperphagia, disproportionate hyperinsulinemia, early speech, and language delay with frequent good resolution, behavioral problems
Kinase suppressor of Ras 2 (KSR2)	Mild hyperphagia and reduced basal metabolic rate, insulin resistance acanthosis nigricans is a frequent feature, irregular menses, early development of type 2 diabetes mellitus
Syndromic conditions	
Prader-Willi syndrome	Hypotonia and failure to thrive in infancy with subsequent weight gain, short stature, hyperphagia, hypogonadotropic hypogonadism, developmental delay
Albright hereditary osteodystrophy	Skeletal defects, short stature may be present impaired olfaction, developmental delay and hormone resistance (e.g., parathyroid hormone) if mutation is maternally derived
Bardet-Biedl syndrome	Dysmorphic extremities (syndactyly/brachydactyly/polydactyly), retinal dystrophy or pigmentary retinopathy, renal abnormalities, hypogonadism, developmental delay
Alstrom syndrome	Retinal dystrophy; extreme insulin resistance; deafness; dilated cardiomyopathy; progressive pulmonary, hepatic, and renal dysfunctions
Endocrinopathies	
Hypothyroidism Cushing syndrome, Hypothalamic obesity, Growth hormone deficiency Persistent hyperinsulinism	

Source: modified from references (7, 9).

GLOMERULAR FILTRATION RATE DETERMINATION

Urine is the product of ultrafiltration of blood plasma through a multilayered structure known as glomerular filter, which is composed, by the fenestrated endothelium, glomerular basement membrane, and glomerular epithelium or podocyte layer (20). The glomerular filtration rate (GFR) can be expressed as the volume of plasma that can be completely cleared of a substance in a time unit and is usually expressed as milliliters per minute (ml/min). To facilitate comparison of GFR among children and adults, body surface standardization to a body surface area of $1.73m^2$ is used, and GFR is expressed as ml/min/$1.73m^2$. The evaluation of GFR in children, should also take into consideration the postnatal kidney maturation which leads to an increase in GFR from low gestational age- dependent levels at birth to the adult level of 120 mL/min/1.73 m^2 which is reached at ∼2 years of age (21).

The total kidney glomerular filtration rate (GFR) is determined by the sum of nephrons and the GFR within each nephron or single nephron GFR A decline in kidney function can be secondary to a decrease in SNGFR because of systemic conditions such as hypoperfusion, and/or by a reduction in nephron number (21).

The simplified functional pore model is the most frequently utilized model to describe the filtration process. The sieving coefficient, which is the ratio of the concentration of each molecule in the urine and in plasma, characterizes the filtration of molecules of different sizes (22). As the sieving coefficients for small molecules <1 kDa are 1, the substances belonging to this group that, contrary to creatinine, do not undergo tubular reabsorption or secretion, such as 51Cr- EDTA (0.34 kDa), iohexol (0.82 kDa), 125I-iothalamate (0.64 kDa) are frequently used to measure GFR. Inulin, an inactive, uncharged 5.2 kDa polymer of fructose, presents many of the characteristics of an ideal marker and its clearance is to date the gold standard for this measurement (21–23).

The plasma disappearance or the urinary excretion of an intravenous injection of a non-radioactive low molecular weight substance such as Iohexol, is particularly suitable to evaluate GFR in children and fertile women (23). However, in the interest of patient comfort, time, and cost, in clinical practice, GFR is generally calculated by *GFR estimating equations* based upon the plasma levels of creatinine (SCr), cystatin C (sCysC) or both. The measured value of plasma creatinine is strongly influenced by non -GFR factors such as muscle mass, growth, diet, and illness, by its tubular and gastrointestinal secretion and by the problems associated with the lack of standardization of its laboratory assay. As a consequence, SCr is considered a suboptimal GFR biomarker (21). Given the limitations of SCr, creatinine-based formulas include surrogates for muscle mass, such as height, weight, and gender, which is especially useful in children and adolescents, as in the pediatric age range, the level of SCr corresponding to normal kidney function /GFR, varies with age (21).

The first pediatric GFR formulas were derived independently by Schwartz and Counahan-Barratt in the mid-1970's. Although both equations were developed in children with chronic renal disease, and use height as a surrogate for growth, they present different proportionality constants reflecting the relationship between urinary creatinine excretion and body size. The original Schwartz formula yields a constant at 0.55, has been derived using a modified Jaffe creatinine assay and inulin clearance as the reference standard (24, 25). The Counahan Barratt formula, uses the plasma clearance of intravenously administered 51-chromium edetic acid (5"Cr-EDTA) as reference standard and yields a proportionality constant of 0.43. It has been derived using creatinine determination by Jaffe reaction, after adsorption by an ion-exchange resin to remove non-creatinine chromogens (26).

The Jaffe assay, an alkaline picrate colorimetric reaction, is affected by the presence of interfering, non-creatinine chromogens, which may falsely elevate SCr by up to 20%, especially at lower levels of creatinine, as observed in the 1st year of life (25). An enzymatic assay for creatinine determination has been developed resulting in 20–30% lower creatinine levels and which are more consistent with accurate HPLC- derived creatinine values (27, 28). To enable the use of the less expensive Jaffe method for creatinine determination in lower resource areas, a correction for a constant bias as compared to the Isotope Dilution Mass Spectrometry (IDMS) reference method has been introduced, accounting for the contribution of serum protein to Jaffe's creatinine, yielding a creatinine value closer to that obtained using an enzymatic creatinine assay (29). Use of an IDMS-traceable creatinine value with the original Schwartz equation will overestimate GFR by 20–40% (21).

More recently, more precise creatinine-based GFR estimation have yielded full - age -spectrum (FAS) equations, such as the Lund–Malmo revised (LMR) equation LMR18 and the new European Kidney Function Consortium (EKFC) equation, valid for both children and adults (30, 31). The development of FAS equations provide a seamless definition of normality values of GFR progression from adolescence to adulthood and from adulthood to old age. Their utilization may yield age- specific GFR values and an age -adapted definition of chronic renal disease, in consonance with morbidity and mortality data, which may result in a much lower global chronic kidney disease prevalence particularly for elderly individuals (32). In young adults, 18–26 years of age, eGFR may be underestimated with the pediatric CKiD formula (28) and overestimated by the adult CKD- EPI equation (33). Of note, averaging results from the pediatric and adult formulas yields an eGFR result which is close to the one obtained by an iohexol GFR determination (21).

Serum cystatin C (sCys-C), a 13 kDa cationic cysteine protease inhibitor is produced, by all nucleated cells, at a constant rate. sCys-C is freely filtered by the glomerulus; it does not undergo tubular secretion and is reabsorbed and metabolized by the proximal tubule epithelial cells, high concentrations of CysC can be demonstrated in the urine from patients with renal tubular dysfunction, due to defective reabsorption (34, 35). Unlike creatinine, sCys-c plasma levels are mainly determined by GFR, without significant influence of age, gender, and muscle mass. s-Cys-c does not seem to be trans-placentally exchanged, In children, sCys-c highest concentration occurs on the 1st day of life. s-CysC levels are higher in preterm than full-term infants, rapidly decreasing during the next months of life. s CysC levels tends to stabilize by ∼1.5–2 years of age (21, 36) or 3 years of age (37). sCys-C levels may be elevated by systemic inflammation, therapeutic use of corticosteroids and by hyperthyroid states, while sCys-C reduced levels may accompany hypothyroid conditions (35). These characteristics suggest. sCys-C as an ideal biomarker of kidney function in children and, in selected clinical situations with altered body composition or reduced muscle mass, such as malnutrition and neuromuscular disease (34).

Reference values for sCys-C obtained in a selected population of healthy individuals, by nephelometry were 0.75+/−0.089 mg/l for children aged 4–19 years, 0.74+/−0.100 mg/l for males, and 0.65+/−0.085 mg/l for females (aged 20–59 years), and 0.83+/-0.103 mg/l for older individuals (> or =60 years) (38).

Of note, sCysC independence of muscle mass enables the use of the same s- Cys C-based GFR estimating equation for children and adults (39). The Caucasian-Asian-Pediatric-Adult (CAPA) equation can be utilized for all individuals above 1 year of age and differently from creatinine-based equations, does not require inclusion of sex and "race" variables, to compensate for differences in muscle mass between individuals (40).

Interestingly, GFR estimates based on sCys-C equations do not offer precision advantages in comparison with

creatinine-based estimates, probably because unmeasured and unidentified non-GFR determinants of cystatin C are as important as those of creatinine. Equations that combine creatinine and sCys-C offer, in general, the most accurate estimate of GFR across the range of GFRs and in subgroups based on demographic and clinical characteristics. This finding may be explained because although errors due to non-GFR determinants of creatinine and sCys-C, are still present in combined equations, their effect is minimized in an equation that uses both markers simultaneously (41). GFR estimating equations combining sCys-C and creatinine have been generated for children (42–44) and adults (41, 45).

The Chronic Kidney Disease in Children study, is a cohort of ∼600 children with chronic kidney disease (CKD) in the United States and Canada. The study focuses on the identification of risk factors for GFR decline and renal disease progression, cardiovascular morbidity, growth failure, and neurocognitive impairment. Utilizing the methodology of iohexol plasma disappearance (iGFR) as a basis for the development of GFR estimating equations, the CKiD study has created the updated version of the bedside creatinine-based GFR equation (21, 28), which is recommended by the National Kidney Disease Education Program for use when creatinine is measured using IDMS methods., as well as, a bedside Cystatin C based GFR equation (21) and a combined GFR equation which include serum creatinine, blood urea nitrogen, height, gender, and cystatin C (21, 44). The study initially chose to measure sCys-C by an immunoturbidimetric method, which provided technically inferior results, motivating the change to immunonephelometry. The reciprocal of immunonephelometric cystatin C correlated as well with GFR as height/ serum creatinine ratio (both 0.88). The resulting combined equation, shown among others, in **Table 3**, is highly accurate and precise, providing good results in children with CKD in a GFR range from 15 to 75 ml/min per 1.73 m^2. It is being utilized to estimate GFR in the follow up of the CKiD cohort in the clinical visits when iohexol is not administered. eGFR equations perform best when applied with the same laboratory methodology and patient population characteristics that were used in its development. The new Schwartz equations were developed using data from pediatric patients with chronic kidney disease and an isotope dilution mass spectrometry (IDMS)-traceable enzymatic creatinine method. Studies to establish its applicability to children of normal growth and muscle mass, and higher GFR are needed (44).

Of note, the arithmetic mean of the results of a cystatin C based GFR estimating equation and a creatinine-based estimating equation has been proven to perform as well as, or even better, for adults and children, than complex combined equations (51–55).

The most frequently used GFR determination equations are the updated Schwartz creatinine formula in children (CKiD) (28) and the CKD-EPI Creatinine Equation in adults (41). **Tables 3**, **4** present respectively, a list of some of the pediatric and adult GFR estimating equations. **Table 3** includes the Pottel height- independent GFR estimation equation, based on levels of enzymatic / isotope dilution mass spectrometry (IDMS) – equivalent assay for serum creatinine, in mg/dL (46, 47), whose performance is comparable to the height-dependent (Schwartz) equations in the identification of renal dysfunction (GFR < 75 mL/min/1.73 m^2) in children (46). **Table 4** focuses on the CKD-EPI (56) and Modification of Diet in Renal Disease (MDRD) based on serum creatinine values standardized to isotope dilution mass spectroscopy (IDMS), devised for adult individuals. Of note the MDRD Study equation despite its

TABLE 3 | Creatinine- based, cystatin based and combined creatinine/cystatin GFR Estimating Equations (ml/min/1.73 m^2) that have been developed for use in pediatric individuals.

CREATININE—BASED GFR ESTIMATING EQUATIONS	
Schwartz "bedside (original- 1976) in mL/min/1.73 m^2 (24, 25)	eGFR = k × L (cm)/PCr (mg/dL) where k ∼ 0.33 (preterm infant), k ∼ 0.45 (full term), k ∼ 0.55 (children and adolescent females), k ∼ 0.7 (adolescent males)
Counahan-Barrat in mL/min/1.73 m^2 (26)	eGFR = 0.43 × L (cm)/PCr (mg/dL)
Updated Schwartz (CKiDCr in mL/min/1.73 m^2) (28)	eGFR = 0.413 × L (cm)/PCr (mg/dL)
Pottel Belgium/Pottel Lyon Equation in mL/min/1.73 m^2 (46, 47)	eGFR = 107.3 /(SCr/Q) where Q is median serum creatinine concentration of the average healthy individual in that population. based on age and sex (for Q values up to 20 years of age see **Table 5**)

New Pottel equation

	Age	SCr/Q	Equation
New Pottel et al. (31)	2–40 yr	<1	**$107.3 \times (SCr/Q)^{-0.322}$**
		≥1	**$107.3 \times (SCr/Q)^{-1.132}$**
	> 40 yr	<1	**$107.3 \times (SCr/Q)^{-0.322} \times 0.990^{(Age-40)}$**
		≥1	**$107.3 \times (SCr/Q)^{-1.132} \times 0.990^{(Age-40)}$**

CYSTATIN C - BASED GFR ESTIMATING EQUATIONS	
CKiDCys-C (Schwartz "bedside" cystatin C) in mL/min/1.73 m^2 (21)	$eGFR = 70.69 \times [\text{cystatin C (mg/L)}]^{-0.931}$
CAPA Equation (>1 year of age) in in mL/min/1.73 m^2 (40)	$eGFR=130 \times \text{cystatin C}^{-1.069} \times \text{age}^{-0.117}-7$
Larsson (ml/min) (48)	$eGFR = 77.24 \times (Cys^{-1.2623})$
Le Bricon (in mL/min/1.73 m^2) (49)	eGFR= (78/Cys) + 4
Filler (in mL/min/1.73 m^2) (50)	Log(eGFR) = 1.962 + [1.123 × log(1/cystatin)]
Zappitelli ((ml/min pe/ 1.73 m^2) (43)	$eGFR = 75.94 \times Cys^{-1.17}$

COMBINED CREATININE/CYSTATIN C GFR ESTIMATING EQUATIONS	
CKiD (44)	$eGFR = 39.8*[ht(m)/Scr]^{0.456}[1.8/cysC]^{0.418}[30/BUN]^{0.079}1.076^{gender}[ht(m)/1.4]^{0.179}$ gender 1 male; 0 female
Zappitelli combined creatinine—cystatin C (ml/min / 1.73 m^2) (43)	$eGFR = (507.76 \times e^{0.003 \times height)}/(Cys^{0.635} \times Cr^{0.547}) \times 1.165$ if renal transplant
Bouvet (in ml/min) (42)	$eGFR = 63.2 \times (Cr/96)^{-0.35} \times (Cys/1.2)^{-0.56} \times (weight/45)^{0.30} \times (age/14)^{0.40}$

eGFR, estimated GFR L ∼ length/height in cm; PCr/SCr serum creatinine in mg/dL, Cystatin C in mg/L.

TABLE 4 | Creatinine Equation (CKD-EPI 2009), Cystatin C Equation (CKD-EPI 2012), and Creatinine–Cystatin C Equation (CKD-EPI 2012) for Estimating GFR, Expressed for Specified Sex, Serum Creatinine Level, and Serum Cystatin C Level (41) and MDRD equation (56) based on SCr values standardized to isotope dilution mass spectroscopy (IDMS) developed for use in adult individuals.

		Serum Creatinine (SCr) (mg/dL)	
CKD-EPI creatinine equation	Female	≤ 0.7	$144 \times (Scr/0.7)^{-0.329} \times 0.993^{Age}$ [× 1.159 if African American]
	Female	>0.7	$144 \times (Scr/0.7)^{-1.209} \times 0.993^{Age}$ [× 1.159 if African American]
	Male	≤0.9	$141 \times (Scr/0.9)^{-0.411} \times 0.993^{Age}$ [× 1.159 if African American]
	Male	>0.9	$141 \times (Scr/0.9)^{-1.209} \times 0.993^{Age}$ [× 1.159 if African American]
		Serum Cystatin C (SCys) (mg/L)	
CKD-EPI Cystatin C equation	Female or male	≤*0.8*	$133 \times (Scys/0.8)^{-0.499} \times 0.996^{Age}$ [× 0.932 if female]
	Female or Male	>0.8	$133 \times (Scys/0.8)^{-1.328} \times 0.996^{Age}$ [× 0.932 if female]

		SCr	SCys	
CKD-EPI creatinine–cystatin C equation	Female	≤ 0.7	≤0.8	$130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if African American]
			>0.8	$130 \times (Scr/0.7)^{-0.248} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if African American]
	Female	>0.7	≤0.8	$130 \times (Scr/0.7)^{-0.601} \times (Scys/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if African American]
			>0.8	$130 \times (Scr/0.7)^{-0.601} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if African American]
	Male	≤0.9	≤0.8	$135 \times (Scr/0.9)^{-0.207} \times (Scys/0.8^{)-0.375} \times 0.995^{Age}$ [× 1.08 if African American]
			>0.8	$135 \times (Scr/0.9)^{-0.207} \times (Scys/0.8^{)-0.711} \times 0.995^{Age}$ [× 1.08 if African American]
	Male	>0.9	≤0.8	$135 \times (Scr/0.9^{)-0.601} \times (Scys/0.8^{)-0.375} \times 0.995^{Age}$ [× 1.08 if African American]
			>0.8	$135 \times (Scr/0.9^{)-0.601} \times (Scys/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if African American]

MDRD Study equation	Serum creatinine values standardized to isotope dilution mass spectroscopy (IDMS)	$GFR = GFR\ (mL/min/1.73\ m^2) = 175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female) $\times$ (1.212 if African American)

development in individuals with CKD, has gained widespread use. Estimated GFR using this equation is reported by most clinical laboratories when measurement of serum creatinine is ordered, which is inadequate because although the MDRD equation performs well in patients with CKD, it systematically underestimates measured GFR at higher levels such as potential kidney donors, young people with type 1 diabetes and patients with substantially reduced muscle mass (45). **Table 5** presents median serum creatinine concentrations (Q) for pediatric and adult individuals to be used in the height independent GFR estimating equations developed by Pottel et al. (46, 57, 58).

TABLE 5 | Q-values [=median serum creatinine in μmol/L (mg/dL)], according to age or height, for height -independent full age spectrum SCr equation by Pottel et al. (46, 57, 58).

Age, years	Height, cm	Qb, μmol/L (mg/dL)
BOYS AND GIRLS		
1	75.0	23 (0.26)
2	87.0	26 (0.29)
3	95.5	27 (0.31)
4	102.5	30 (0.34)
5	110.0	34 (0.38)
6	116.7	36 (0.41)
7	123.5	39 (0.44)
8	129.5	41 (0.46)
9	135.0	43 (0.49)
10	140.0	45 (0.51)
11	146.0	47 (0.53)
12	152.5	50 (0.57)
13	159.0	52 (0.59)
14	165.0	54 (0.61)
MALE ADOLESCENTS		
15	172.0	64 (0.72)
16	176.0	69 (0.78)
17	178.0	72 (0.82)
18	179.0	75 (0.85)
19	180.0	78 (0.88)
MALE ADULTS		
≥20	≥181.5	80 (0.90)
FEMALE ADOLESCENTS		
15	164.5	57 (0.64)
16	166.0	59 (0.67)
17	166.5	61 (0.69)
18	167.0	61 (0.69)
19	167.5	62 (0.70)
FEMALE ADULTS		
≥20	≥168.0	62 (0.70)

Obs: Height is the median height of a child or adolescent at the specified age (Belgian growth curves).

In neonates sCys-C is a better biomarker of GFR than creatinine. During pregnancy, the placenta is responsible for fetal creatinine equilibration, at birth the neonate SCr reflects the mother's SCr levels, as the neonate's kidney takes control, there is an initial increase in the neonate's SCr followed by a gradual fall along the first 2 years of life when the kidneys reach full maturation. sCys-C levels at birth are elevated on account of the physiologically lower GFR of the neonate. Kidney

maturation leads to increases in GFR causing, sCys-C levels to fall reaching a plateau after 18 months of age (21, 36, 37). A recent multicentric worldwide systematic review, from 10 countries across four continents, described sCysC progression, in 1,468 babies born preterm, along the 1st month of life, infants born at 24–28 weeks of gestational age (GA), presented s- CysC values ranging from 1.44 to 1.90 mg/L, on day 1, to 1.36 to 2.02 between 4 and 30 days of life, while in preterm infants born after 34 weeks of gestational age, sCys C values ranged from 1.22 to 1.96 mg/L, on day 1, to 1.22 to 1.82 between 4 and 30 days of life (59). Mean sCys C values cystatin C, usually fall to a mean concentration of 0.80 mg/liter by the 1st year of life (34).

OBESITY AND THE KIDNEY

The renal effects of obesity can be indirect, *via* development of systemic arterial hypertension and / or diabetes mellitus, or direct, by adipose tissue hypertrophy and its ectopic accumulation in the kidney. Ectopic renal accumulation of lipids lead to multiple functional and structural changes, which although not fully understood, may lead to glomerular hypertension, increased glomerular permeability, hyperfiltration, glomerulomegaly, albuminuria, and in some cases, focal and segmental glomerulosclerosis (FSGS) characterizing the obesity related glomerulopathy (60–63).

The Chronic Kidney Disease Prognosis Consortium (CKD-PC) study evaluated the association between various measures of adiposity with eGFR deterioration and mortality from all causes. The included participants were 18 years and older, eGFR, was calculated utilizing the CKD-EPI equation. BMI data were collected from 39 general population, six high CVD risk, and 18 CKD cohorts, between 1970 and 2017. Participants in higher categories of BMI were more often of black race, more likely to have hypertension, diabetes, and albuminuria, and less likely to be current smokers. After long-term follow-up, individuals with a BMI $>$ 30 kg/m^2 belonging to the cohorts of the general population, showed a significantly higher risk for GFR decline, and a "J" shaped association between BMI and mortality, with the lowest risk for BMI of 25 kg/m^2. In the cohorts with high cardiovascular risk and CKD, the risk association between high BMI and GFR decline was weaker than in the general population, with a "J" shaped association between BMI and death, with the lowest risk for BMI values between 25 and 30 kg/m^2 (64).

A recent multicenter cross-sectional study involving 3,118 youth with overweight /obesity, and 286 healthy normal weight youth between 5 and 14 years of age. compared the prevalence of mildly reduced estimated GFR (eGFR $>$ 60 and $<$ 90 mL/min/1.73 m^2), and its association with cardiometabolic risk factors in overweight/ obese youth. eGFR was calculated using the updated bedside Schwartz equation and Full Age Spectrum (FAS) equation. The FAS equation identified a higher prevalence of youth with mildly reduced estimated GFR, compared to bedside Schwartz equation. Individuals with mildly reduced estimated glomerular filtration rate, according with both equations showed lower birth weight, younger age, higher BMI-SDS, non-high-density lipoprotein-cholesterol and serum uric acid, as compared to those with normal eGFR (65).

The association between BMI and risk of developing all-cause, diabetic, and non-diabetic end stage kidney disease (ESKD) was assessed in 1.2 million adolescents aged 17 years: over 25 years. This was a nation-wide population- based retrospective cohort study that linked medical data of 1,194,704 adolescents aged 17 years who had been examined for fitness for military service, between January 1, 1967, and December 31, 1997, to the Israeli ESRD registry. The overall incidence rate of CKD was 2.87 cases per 100,000 person- years. Compared to adolescents with normal weight, overweight and obese adolescents had an increased future risk for treated end- stage kidney disease (ESKD), with incidence rates of 6.08 and 13.40 cases per 100,000 person-years, respectively. Overweight and obesity were strongly associated with all-cause treated end- stage kidney disease [hazard ratio of 3.00 (95% CI, 2.50–3.60) and 6.89 (95% CI, 5.52–8.59), respectively], as well as, strong and independent risk factors for diabetic and non-diabetic kidney disease (66).

The effect of obesity on the kidney function could be time-dependent. In fact, duration of obesity negatively influences the eGFR in obese children (67) and in children with congenital solitary kidney (68). Moreover, the duration of obesity could increase the risk of development of kidney injury in adults with congenital solitary kidney (69). Obesity is a clinical condition associated with altered body composition. As discussed above, in this context, creatinine may not be the ideal biomarker for GFR measurement. This is especially true in obese children and adolescents, in whom besides the altered body composition; normalization of eGFR to 1.73 m^2, introduces another bias. As BMI is strongly correlated with the body surface, the adjustment for body surface removes the effect of body weight on GFR, causing an underestimation of true GFR in individuals with a higher BMI, masking the installation of hyperfiltration (70). The challenges of measuring GFR in obese pediatric patients can be overcome either by replacing the real weight in the calculation of body surface by the ideal weight, calculated as,

Ideal weight = BMI at 50th percentile (kg/m^2) X Height ^{2} (m) (71), or by using a height -independent creatinine -based equation for GFR estimation. Another possibility would the utilization of a sCysC -based equation for GFR estimation.

Weight-loss interventions slow or reverse early CKD progression, in adults and children (72–74). Pre- bariatric surgery data of 242 adolescents from the "Teen-Labs" study show microalbuminuria in 14%, macroalbuminuria in 3%, serum cystatin C-based eGFR $<$ 60 mL / min / 1.73 m^2 in 3% and eGFR $>$ 150 mL / min / 1.73 m^2 in 7.1%. Lower eGFR was associated with higher values of BMI and/ or HOMA-IR. Three years post-surgery, patients with baseline serum cystatin C-based eGFR under 90 mL/min/1.73 m^2, showed a significant improvement in mean eGFR from 76 to 102 mL/min/1.73 m^2, while participants with albuminuria (albumin-to-creatinine ratio of 30 mg/g and more) at baseline presented a reduction in the geometric mean of ACR from 74 mg/g to 17 mg/g at 3 years follow-up. Patients with normal baseline renal function and no albuminuria at baseline remained stable throughout the study period. Among individuals

with a BMI $\geq$ 40 kg/m^2, increased BMI was associated with a significantly lower eGFR, while no association was observed in those with a BMI under 40 kg/m^2. After adjusted analysis, post-surgical eGFR increased by 3.9 mL/min/1.73 m^2 for each 10-unit loss of BMI (73). Studies in adults, however, show a consistent association between obesity and lower mortality in those with advanced CKD, especially among hemodialysis patients, suggesting that the reverse epidemiology of obesity is biologically reasonable (75).

Final Commentaries

Obesity represents a major risk factor for the development of CKD. Sequential GFR determinations are the basis of renal function follow-up in affected individuals across all age groups. Obesity is also an example of a situation in which due to altered body composition, creatinine based GFR evaluation may lead to imprecise results. Additionally, some of the currently available creatinine GFR determination equations were developed in CKD patients' cohorts, which makes their utilization in individuals with normal GFR amenable to error (21, 28, 56).

Unfortunately, with the exception GFR determinations based on plasma disappearance of an intravenous injection of a low molecular weight substance that, on account of their precision, are routinely used for clinical research, there is no agreement on which is the best GFR determination equation to be used in children, adults or across the life span, as demonstrated by the multiple equations described above. This dilemma is expressed in a recent study, in obese children, in which the detection of changes in eGFR over time in response to therapeutic interventions were detectable only with the utilization of a height independent eGFR equation (74). Of note, another pediatric option of height independent GFR estimation is the equation proposed by the British Columbia Children's Hospital (BCCH; eGFR-BCCH), which in comparison to Potttel's equation might yield inferior results (76).

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Jastreboff AM, Kotz CM, Kahan S, Kelly AS, Heymsfield SB. Obesity as a disease: the obesity society 2018 position statement. *Obesity.* (2019) 27:7–9. doi: 10.1002/oby.22378
2. Liu B, Du Y, Wu Y, Snetselaar LG, Wallace RB, Bao W. Trends in obesity and adiposity measures by race or ethnicity among adults in the United States 2011-18: population-based study. *BMJ.* (2021) 372:n365. doi: 10.1136/bmj.n365
3. WHO Expert Committee. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser*. (1995) 854:1–452.
4. CDC Clinical Growth Charts. *Atlanta, Georgia. Centres for Disease Control and Prevention.* (2000). Available online at: http://www.cdc.gov/growthcharts (accessed August 22, 2021).
5. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ.* (2000) 320:1240–3. doi: 10.1136/bmj.320.7244.1240
6. Wang Y, Wang JQ. A comparison of international references for the assessment of child and adolescent overweight and obesity in different populations. *Eur J Clin Nutr.* (2002) 56:973–82. doi: 10.1038/sj.ejcn.1601415
7. Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric obesity-assessment, treatment, and prevention: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* (2017) 102:709–57. doi: 10.1210/jc.2016-2573
8. Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med.* (2015) 373:1307–17. doi: 10.1056/NEJMoa1502821
9. Aggarwal B, Jain V. Obesity in children: definition, etiology and approach. *Indian J Pediatr.* (2018) 85:463–71. doi: 10.1007/s12098-017-2531-x
10. Qiao Q, Nyamdorj R. The optimal cut-off values and their performanceof waist circumference and waist-to-hip ratio for diagnosing type II diabetes. *Eur J Clin Nutr.* (2010) 64:23–9. doi: 10.1038/ejcn.2009.92
11. Khadilkar A, Ekbote V, Chiplonkar S, Khadilkar V, Kajale N, Kulkarni S, et al. Waist circumference percentiles in 2-18 year old Indian children. *J Pediatr.* (2014) 164:1358–62.e2. doi: 10.1016/j.jpeds.2014.02.018
12. Ashwell M, Gibson S. Waist-to-height ratio as an indicator of 'early health risk': simpler and more predictive than using a 'matrix' based on BMI and waist circumference. *BMJ Open.* (2016) 6:e010159. doi: 10.1136/bmjopen-2015-010159
13. Mehta SK. Waist circumference to height ratio in children and adolescents. *Clin Pediatr*. (2015) 54:652–8. doi: 10.1177/0009922814557784
14. Browning LM, Hsieh SD, Ashwell M. A systematic review of waist-to height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. *Nutr Res Rev.* (2010) 23:247–69. doi: 10.1017/S0954422410000144
15. Archer E, Lavie CJ, Hill JO. The contributions of 'diet', 'genes', and physical activity to the etiology of obesity: contrary evidence and consilience progress in cardiovascular diseases. *Progr. Cardiovasc. Dis.* (2018) 61:89–102. doi: 10.1016/j.pcad.2018.06.002
16. Church TS, Thomas DM, Tudor-Locke C, Earnest CP, Rodarte RQ, Martin CK, et al. Trends over 5 decades in US occupation-related physical activity and their associations with obesity. *PLoS ONE.* (2011) 6:e19657. doi: 10.1371/journal.pone.0019657
17. Khandelwal P, Jain V, Gupta AK, Kalaivani M, Paul VK. Association of early postnatal growth trajectory with body composition in term low birth weight infants. *J Dev Orig Health Dis.* (2014) 5:189–96. doi: 10.1017/S2040174414000178
18. Di Angelantonio E, Bhupathiraju ShN, Wormser D, Gao P, Kaptoge S, Berrington de Gonzalez A, et al. Body-mass index and all-cause mortality: individual- participant-data meta-analysis of 239 prospective studies in four continents. *Lancet.* (2016) 388:776–86.
19. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med.* (2011) 365:1876–85. doi: 10.1056/NEJMoa1010112
20. Haraldsson B, Nystrom J, Deen WM. Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev.* (2008) 88:451–87. doi: 10.1152/physrev.00055.2006
21. Mian AN, Schwartz GJ. Measurement and estimation of glomerular filtration rate in children. *Adv Chronic Kidney Dis.* (2017) 24:348–56. doi: 10.1053/j.ackd.2017.09.011
22. Norden AGW, Lapsley M, Lee PJ, Pusey CD, Scheinman SJ, Tam FWK, et al. Glomerular protein sieving and implications for renal failure in Fanconi syndrome. *Kidney Int.* (2001) 60:1885–92 1755.2001.00016.x. doi: 10.1046/j.1523-1755.2001.00016.x
23. Soveri I, Berg U, Björk J, Elinder CG, Grubb A, Mejare I, et al. Measuring GFR: a systematic review. *Am J Kidney Dis.* (2014) 64:411–24. doi: 10.1053/j.ajkd.2014.04.010
24. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics.* (1976) 58:259–63.

25. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. *Pediatr Clin North Am.* (1987) 34:571–90. doi: 10.1016/S0031-3955(16)36251-4
26. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM. Estimation of glomerular filtration rate from plasma creatinine concentration in children. *Arch Dis Child.* (1976) 51:875–78. doi: 10.1136/adc.51.11.875
27. Schwartz GJ, Kwong T, Erway B, Warady B, Sokoll L, Hellerstein S, et al. Validation of creatinine assays utilizing HPLC and IDMS traceable standards in sera of children. *Pediatr Nephrol.* (2009) 24:113–9. doi: 10.1007/s00467-008-0957-0
28. Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol.* (2009) 20:629–37. doi: 10.1681/ASN.2008030287
29. Speeckaert MM, Wuyts B, Stove V, Walle JV, Delanghe JR. Compensating for the influence of total serum protein in the Schwartz formula. *Clin Chem Lab Med.* (2012) 50:1597–600. doi: 10.1515/cclm-2012-0033
30. Björk J, Nyman U, Delanaye P, Grubb A, Larsson A, Vranken L, et al. A novel method for creatinine adjustment makes the revised Lund-Malmö GFR estimating equation applicable in children. *Scand J Clin Lab Invest.* (2020) 80:456–63. doi: 10.1080/00365513.2020.1774641
31. Pottel H, Björk J, Courbebaisse M, Couzi L, Ebert N, Eriksen BO, et al. Development and validation of a modified full age spectrum creatinine- based equation to estimate glomerular filtration rate: A cross-sectional analysis of pooled data. *Ann Intern Med.* (2021) 174:183–91. doi: 10.7326/M20-4366
32. Delanaye P, Jager KJ, Bökenkamp A, Christensson A, Dubourg L, Eriksen BO, et al. CKD: a call for an age-adapted definition. *J Am Soc Nephrol.* (2019) 30:1785–805. doi: 10.1681/ASN.2019030238
33. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
34. Finney H, Newman DJ, Thakkar H, Fell JM, Price CP. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. *Arch Dis Child.* (2000) 82:71–5. doi: 10.1136/adc.82.1.71
35. Andersen TB, Eskild-Jensen A, Frøkiaer J, Brøchner-Mortensen J. Measuring glomerular filtration rate in children; can cystatin C replace established methods? *A review Pediatr Nephrol.* (2009) 24:929–41. doi: 10.1007/s00467-008-0991-y
36. Fischbach M, Graff V, Terzic J, Bergere V, Oudet M, Hamel G. Impact of age on reference values for serum concentration of cystatin C in children. *Pediatr Nephrol.* (2002) 17:104–6. doi: 10.1007/s00467-001-0777-y
37. Harmoinen A, Ylinen E, Ala-Houhala M, Janas M, Kaila M, Kouri T. Reference intervals for cystatin C in pre- and full-term infants and children. *Pediatr Nephrol.* (2000) 15:105–8. doi: 10.1007/s004670000421
38. Galteau MM, Guyon M, Gueguen R, Siest G. Determination of serum cystatin C: biological variation and reference values. *Clin Chem Lab Med.* (2001) 39:850–7. doi: 10.1515/CCLM.2001.141
39. Bökenkamp A, Domanetzki M, Zinck R, Schuman G, Byrd D, Brodehl J. Cystatin C – a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics.* (1998) 101:875–81. doi: 10.1542/peds.101.5.875
40. Grubb A, Horio M, Hansson LO, Björk J, Nyman U, Flodin M, et al. Generation of a new cystatin C-based estimating equation for glomerular filtration rate by use of 7 assays standardized to the international calibrator. *Clin Chem.* (2014) 60:974–86. doi: 10.1373/clinchem.2013.220707
41. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* (2012) 367:20–9. doi: 10.1056/NEJMoa1114248
42. Bouvet Y, Bouissou F, Coulais Y, Séronie-Vivien S, Tafani M, Decramer S, et al. GFR is better estimated by considering both serum cystatin C and creatinine levels. *Pediatr Nephrol.* (2006) 21:1299–306. doi: 10.1007/s00467-006-0145-z
43. Zappitelli M, Parvex P, Joseph L, Paradis G, Grey V, Lau S, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. *Am J Kidney Dis.* (2006) 48:221–30. doi: 10.1053/j.ajkd.2006.04.085
44. Schwartz GJ, Schneider MF, Maier PS, Moxey-Mims M, Dharnidharka VR, Warady BA, et al. Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. *Kidney Int.* (2012) 82:445–53. doi: 10.1038/ki.2012.169
45. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis.* (2008) 51:395–406. doi: 10.1053/j.ajkd.2007.11.018
46. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol.* (2012) 27:973–9. doi: 10.1007/s00467-011-2081-9
47. De Souza V, Pottel H, Hoste L, Dolomanova O, Cartier R, Selistre L, et al. Can the height-independent Pottel eGFR equation be used as a screening tool for chronic kidney disease in children? *Eur J Pediatr.* (2015) 174:1225–35. doi: 10.1007/s00431-015-2523-7
48. Larsson A, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. *Scand J Clin Lab Invest.* (2004) 64: 25–30. doi: 10.1080/00365510410003723
49. Le Bricon T, Thervet E, Froissart M, Benlakehal M, Bousquet B, Legendre C, et al. Plasma cystatin C is superior to 24-h creatinine clearance and plasma creatinine for estimation of glomerular filtration rate 3 months after kidney transplantation. *Clin Chem.* (2000) 46: 1206–7. doi: 10.1093/clinchem/46.8.1206
50. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? *Pediatr Nephrol.* (2003) 18:981–5. doi: 10.1007/s00467-003-1271-5
51. Nyman U, Grubb A, Sterner G, Björk J. Different equations to combine creatinine and cystatin C to predict GFR. Arithmetic mean of existing equations performs as well as complex equations. *Scand J Clin Lab Invest.* (2009) 69:619–27. doi: 10.1080/00365510902946992
52. Grubb A, Nyman U, Björk J. Improved estimation of glomerular filtration rate (GFR) by comparison of eGFR cystatin C and eGFR creatinine. *Scand J Clin Lab Invest.* (2012) 72:73–7. doi: 10.3109/00365513.2011.634023
53. den Bakker E, Gemke R, van Wijk JAE, Hubeek I, Stoffel-Wagner B, Grubb A, et al. Accurate eGFR reporting for children without anthropometric data. *Clin Chim Acta.* (2017) 474:38–43. doi: 10.1016/j.cca.2017.09.004
54. Leion F, Hegbrant J, den Bakker E, Jonsson M. Abrahamson, Nyman U, et al. Estimating glomerular filtration rate (GFR) in children The average between a cystatin C- and a creatinine-based equation improves estimation of GFR in both children and adults and enables diagnosing Shrunken Pore Syndrome. *Scand J Clin Lab Invest.* (2017) 77:338–44. doi: 10.1080/00365513.2017.1324175
55. den Bakker E, Gemke R, van Wijk JAE, Hubeek I, Stoffel-Wagner B, Bökenkamp A. Combining GFR estimates from cystatin C and creatinine—what is the optimal mix? *Pediatr Nephrol.* (2018) 33:1553–63. doi: 10.1007/s00467-018-3973-8
56. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renaldisease study equation for estimating glomerular filtration rate. *Ann Intern Med.* (2006) 145:247–54. doi: 10.7326/0003-4819-145-4-200608150-00004
57. Pottel H, Vrydags N, Mahieu B, Vandewynckele E, Croes K, Martens F. Establishing age/sex-related serum creatinine reference intervals from hospital laboratory data based on different statistical methods. *Clin Chim Acta.* (2008) 396:49–55. doi: 10.1016/j.cca.2008.06.017
58. Pottel H, Hoste L, Dubourg L, Ebert N, Schaeffner E, Eriksen BO, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol dial transpl.* (2016) 31:798–806. doi: 10.1093/ndt/gfv454
59. Renganathan A, Warner BB, Tarr PI, Dharnidharka VR. The progression of serum cystatin C concentrations within the first month of life after preterm birth-a worldwide systematic review. *Pediatr Nephrol.* (2021) 6:1709–18. doi: 10.1007/s00467-020-04543-1
60. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw.* (2006) 17:4–12.
61. de Vries AP, Ruggenenti P, Ruan XZ, Praga M, Cruzado JM, Bajema IM, et al. ERA-EDTA Working Group Diabesity. Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. *Lancet Diabetes Endocrinol.* (2014) 2:417–26. doi: 10.1016/S2213-8587(14)70065-8

62. Zhu Q, Scherer PE. Immunologic and endocrine functions of adipose tissue: implications for kidney disease. *Nat Rev Nephrol.* (2018) 14:105–20. doi: 10.1038/nrneph.2017.157
63. D'Agati VD, Chagnac A, de Vries AP, Levi M, Porrini E, Herman-Edelstein M, et al. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat Rev Nephrol.* (2016) 12:453–71. doi: 10.1038/nrneph.2016.75
64. Chang AR, Grams ME, Ballew SH, Bilo H, Correa A, Evans M, et al. Adiposity and risk of decline in glomerular filtration rate: meta-analysis of individual participant data in a global consortium. *BMJ.* (2019) 364:k5301. doi: 10.1136/bmj.k5301
65. Di Bonito P, Licenziati MR, Campana G, Chiesa C, Pacifico L, Manco M, et al. Prevalence of mildly reduced estimated GFR by height- or age-related equations in young people with obesity and its association with cardiometabolic risk factors. *J Ren Nutr*. (2021) 2021:S1051-227630285-5. doi: 10.1053/j.jrn.2020.11.005
66. Vivante A, Golan E, Tzur D, Leiba A, Tirosh A, Skorecki K, et al. Body mass index in 12 million adolescents and risk for end-stage renal disease. *Arch Intern Med.* (2012) 172:1644–50. doi: 10.1001/2013.jamainternmed.85
67. Marzuillo P, Grandone A, Di Sessa A, Guarino S, Diplomatico M, Umano GR, et al. Anthropometric and biochemical determinants of estimated glomerular filtration rate in a large cohort of obese children. *J Ren Nutr.* (2018) 28:359–62. doi: 10.1053/j.jrn.2018.01.001
68. La Scola C, Guarino S, Pasini A, Capalbo D, Liguori L, Di Sessa A, et al. Effect of body mass index on estimated glomerular filtration rate levels in children with congenital solitary kidney: a cross-sectional multicenter study. *J Ren Nutr.* (2020) 30:261–67. doi: 10.1053/j.jrn.2019.07.003
69. Marzuillo P, Guarino S, Di Sessa A, Rambaldi PF, Reginelli A, Vacca G, et al. Congenital solitary kidney from birth to adulthood. *J Urol.* (2021) 205:1466–75. doi: 10.1097/JU.0000000000001524
70. Wuerzner G, Bochud M, Giusti V, Burnier M. Measurement of glomerular filtration rate in obese patients: pitfalls and potential consequences on drug therapy. *Obes Facts.* (2011) 4:238–43. doi: 10.1159/000329547
71. Correia-Costa L, Schaefer F, Afonso AC, Bustorff M, Guimarães JT, Guerra A, et al. Normalization of glomerular filtration rate in obese children. *Pediatr Nephrol.* (2016) 31:1321–8. doi: 10.1007/s00467-016-3367-8
72. Xiao N, Jenkins TM, Nehus E, Inge TH, Michalsky MP, Harmon CMH, et al. Kidney function in severely obese adolescents undergoing bariatric surgery. *Obesity.* (2014) 22:2319–25. doi: 10.1002/oby.20870
73. Nehus EJ, Khoury JC, Inge TH, Xiao N, Jenkins TM, Moxey-Mims MM, et al. Kidney outcomes three years after bariatric surgery in severely obese adolescents. *Kidney Int.* (2017) 91:451–8. doi: 10.1016/j.kint.2016.09.031
74. van Dam M, Rijks J, Dorenbos E, Horuz F, van Dael K, Vreugdenhil A. The effect of one year lifestyle intervention on eGFR in children and adolescents with overweight, obesity and morbid obesity. *Sci Rep.* (2019) 9:4504. doi: 10.1038/s41598-019-40767-4
75. Rhee CM, Ahmadi SF, Kalantar-Zadeh K. The dual roles of obesity in chronic kidney disease: a review of the current literature. *Curr Opin Nephrol Hypertens.* (2016) 25:208–16. doi: 10.1097/MNH.0000000000000212
76. Blufpand HN, Westland R, van Wijk JA, Roelandse-Koop EA, Kaspers GJ, Bökenkamp A. Height-independent estimation of glomerular filtration rate in children: an alternative to the Schwartz equation. *J Pediatr.* (2013) 163:1722–7. doi: 10.1016/j.jpeds.2013.08.031

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

MINI REVIEW
published: 23 November 2021
doi: 10.3389/fmed.2021.765924

Chronic Kidney Disease and Arterial Stiffness: A Two-Way Path

Felipe Inserra[1,2]*, *Pedro Forcada*[2,3], *Agustina Castellaro*[4] and *Carlos Castellaro*[2,5]

[1] Advisor of Academic Vice-Rectory Department, Maimonides University, Buenos Aires, Argentina, [2] Master Vascular Mechanics and Arterial Hypertension, Postgraduate Department, Hypertension, Austral University, Buenos Aires, Argentina, [3] Non-Invasive Vascular Labs, CardioArenales and Diagnóstico Integral Médico (DIM) Prevención Cardiovascular, Buenos Aires, Argentina, [4] Pediatric Medicine of Prof. Dr. Juan P Garrahan Hospital, Buenos Aires, Argentina, [5] Department of Nephrology, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC) Hospital, Buenos Aires, Argentina

The kidney-heart relationship has raised interest for the medical population since its vast and complex interaction significantly impacts health. Chronic kidney disease (CKD) generates vascular structure and function changes, with significant hemodynamic effects. The early arterial stiffening in CKD patients is a consequence of the interaction between oxidative stress and chronic vascular inflammation, leading to an accelerated deterioration of left ventricular function and alteration in tissue perfusion. CKD amplifies the inflammatory cascade's activation and is responsible for altering the endothelium function, increasing the vascular tone, wall thickening, and favors calcium deposits in the arterial wall. Simultaneously, the autonomic imbalance, and alteration in other hormonal systems, also favor the overactivation of inflammatory and fibrotic mediators. Thus, hormonal disarrangement also contributes to structural and functional lesions throughout the arterial wall. On the other hand, a rise in arterial stiffening and volume overload generates high left ventricular afterload. It increases the left ventricular burden with consequent myocardial remodeling, development of left ventricular hypertrophy and, in turn, heart failure. It is noteworthy that reduction in glomerular mass of renal diseases generates a compensatory glomerular filtration overdriven associated with large-arteries stiffness and high cardiovascular events. Furthermore, we consider that the consequent alterations of the arterial system's mechanical properties are crucial for altering tissue perfusion, mainly in low resistance. Thus, increasing the knowledge of these processes may help the reader to integrate them from a pathophysiological perspective, providing a comprehensive idea of this two-way path between arterial stiffness and renal dysfunction and their impact at the cardiovascular level.

Keywords: pulse wave velocity (PWV), calcification, CKD progression, pulsatility, cardiovascular events

OPEN ACCESS

Edited by:
Ana Cusumano, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Argentina

Reviewed by:
János Nemcsik, Semmelweis University, Hungary
Nikhil Nair, Akron Children's Hospital, United States

***Correspondence:**
Felipe Inserra
felipe.inserra@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *27 August 2021*
Accepted: *30 September 2021*
Published: *23 November 2021*

Citation:
Inserra F, Forcada P, Castellaro A and Castellaro C (2021) Chronic Kidney Disease and Arterial Stiffness: A Two-Way Path. Front. Med. 8:765924. doi: 10.3389/fmed.2021.765924

INTRODUCTION: THE ROLE OF VASCULAR INJURY IN THE KIDNEY AND CARDIAC DISEASE INTERACTION

A robust functional relationship exists between the kidneys and the cardiovascular system, and this vast and proven interaction has acquired progressive notoriety.

The evidence from population studies clearly shows that renal and cardiac diseases are strongly associated (1, 2). This evidence includes the different clinical situations where structural lesions (tissue, cellular, and subcellular) develop simultaneously with both organs' progressive functional deterioration (3–5).

Of particular interest is the relationship between the heart and kidneys when both are functionally insufficient. This interplay is known as the cardiorenal syndrome, and the most relevant are chronic types 2 and 4 of the original description by Ronco et al. (6). This interaction is also known as "the cardiorenal link" (7).

The pathophysiological and histopathological concepts involved, including the graphics and figures, are designed to understand better the kidney and the heart's role as principals organs involved.

However, these conceptual proposals do not define the precise role or sequence of the macro and microcirculation structures in this process. Therefore, we tried to analyze the process of vascular injury in CKD (8–10), giving the central role it plays and its interaction with different tissue injuries leading to the cardiorenal syndrome's progression. The leading role of the circulation (macro and micro) and its interaction with the kidneys different pathophysiologic hypotheses on intrarenal hemodynamics changes that they generate.

General Concepts of Vascular Behavior

The essential objective of blood traveling through the arterial system is the perfusion and oxygenation of peripheral tissues. Under normal conditions, the cardiac pump discharges the systolic volume (SV) of blood received by the large-caliber elastic arteries, mainly the aorta. However, ∼50% of the previously mentioned SV is dampened due to the compliance of its walls.

The remaining 50% of the SV continues its way to the peripheral arteries. Once the aorta returns to its initial caliber in diastole, its elastic capacity, if it is healthy, sends the remaining volume forward, transforming the arterial flow from pulsatile to continuous in the peripheral circulation. This fact is known as the "Windkessel Phenomena" in comparison to the old fire extinguishing pump.

At the distal level, the arteries have a structural component mainly integrated by smooth muscle cells coupled "in series" with collagen fibers, all influenced by neural and hormonal factors. This massive parallel resistance system is responsible for peripheral vascular resistance and the dissipation of at least two-thirds of the cardiovascular system's pulsatile energy. This fact allows arterioles to adapt to different situations, organs, and pathophysiological circumstances. However, this regulable system loses efficacy because of aging (normal or accelerated) and other conditions—such as high blood pressure, diabetes, and CKD—that cause a decrease in arterial compliance due to the loss of the vessel's elastic components. The half-life of elastin, the main factor responsible for the aorta's elasticity, is measurable in years. Continuous and intermittent distension of the aorta with each heartbeat and during the lifespan causes fatigue and fracture of the elastin fiber, leading to increasing stiffening of the aorta's wall (11). In this scenario, the accumulation of different collagen types that are stiffer than the initial one, and other substances like Advanced Glycation End Products (AGEs) occur, conducting the loss of compliance of the elastic arteries (11, 12). When this loss of arterial compliance, or its opposite, an increase in arterial stiffness, evolve faster than expected by normal aging, we are in the presence of "early vascular aging" (EVA). Certain metabolic disorders and diseases cause this EVA phenomenon to accelerate and appear in earlier stages or with greater severity. Kidney disease is a frequent cause and one of the most representative examples of this phenomenon.

ONE WAY: FROM CHRONIC KIDNEY DISEASE TO VASCULAR INJURY

During the development and progression of CKD, the aortic compliance decreases, reducing SV's buffering capacity, resulting in an exaggerated increase of the systolic pressure (SP) and a drop of the diastolic pressure (DP).

The pulse pressure (PP) or pulsatility is the difference between SP and DP in mmHg. Hence, it is an easy parameter to be observed during the medical examination when measuring blood pressure.

A stiff aorta produces the loss of its properties as a second pump, or "second heart" (a consequence of the lack of elastic recoil of a distensible aorta), with the consequent drop in the diastolic vascular flow and pressure. Aortic stiffness also increases the pulsatility in the peripheral vessels and their irrigated tissues. Due to these conditions, the SV is no longer buffered and continues toward the periphery.

On the other hand, arterial wall stiffening increases the pulse wave velocity (PWV), the speed at which the pulse wave travels through the large arteries' wall, and is currently considered the gold standard for arterial stiffness measurement. It is also an independent prognostic marker of CVE (13, 14). The increase in SP results in increased heart afterload, augmented work of the left ventricle (LF), and increased oxygen consumption by the myocardium. Over time, the persistent imbalance favors the development of left ventricular hypertrophy (LVH) and heart failure (HF).

Another physiological phenomenon is related to the highest speed of the PWV. Under physiological conditions, the incident wave, generated from the systolic discharge, propagates through the arterial system to the reflection points. These sites are the arterial bifurcations or regions of the most significant change in the arterial wall's viscoelastic components. A reflected wave returns in the opposite direction during late systole and adds to the new incident wave, giving rise to the augmentation wave phenomenon, measured as "augmentation index" (Aix) (**Figure 1A**).

In situations of increased arterial stiffness, as occurs in CKD, through mechanisms described in this review, the wave's reflection occurs earlier and arrives prematurely, worsening the Aix, considered an marker of arterial stiffness (**Figure 1B**). Therefore, an additional increase in central aortic pressure (CAP) contributes to increasing cardiac work and oxygen consumption, both of which provoke further activation on the pathways and mechanisms leading to LVH and HF.

A direct relationship stands between renal function impairment with CAP and Aix. Thus, the more significant the CAP and Aix are, the more influential they are on renal function deterioration and death, as was demonstrated by Towsend et al. in the CRIC study (15, 16). Recent data confirmed that Aix was

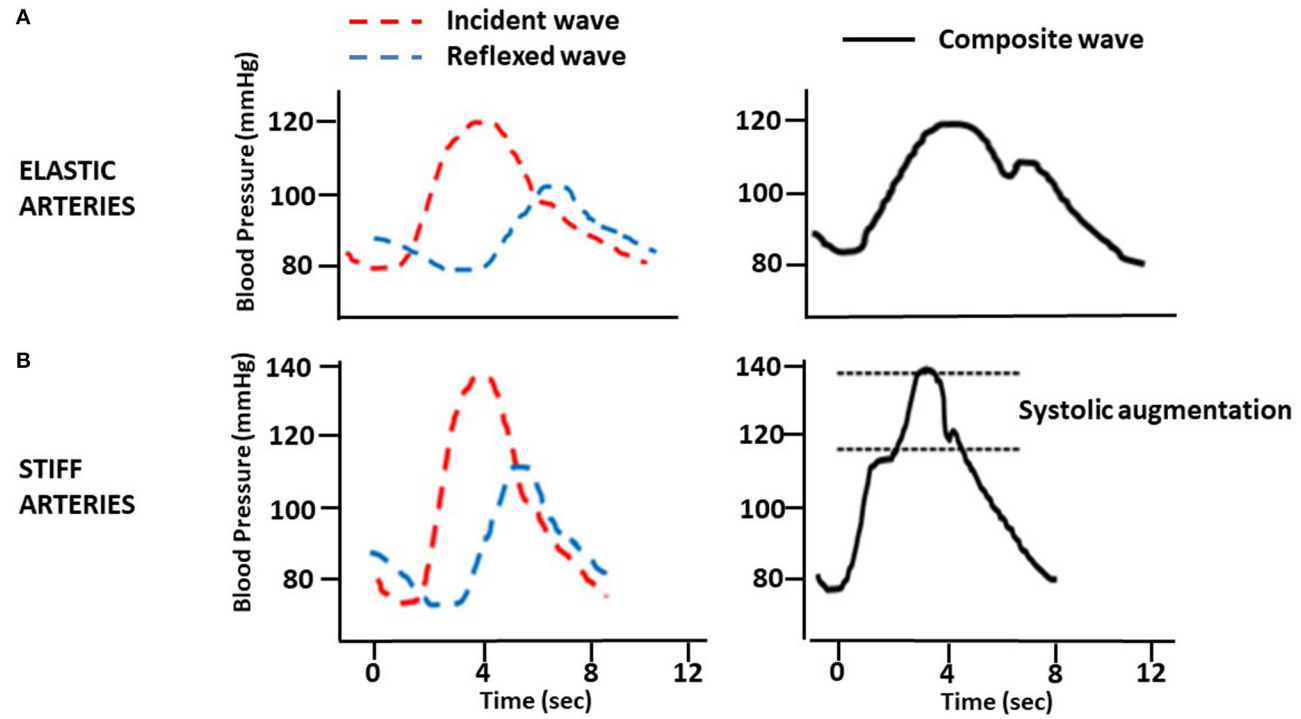


FIGURE 1 | Changes in pulse wave by arterial stiffness. **(A)** Elastic arteries. **(B)** Stiff arteries.

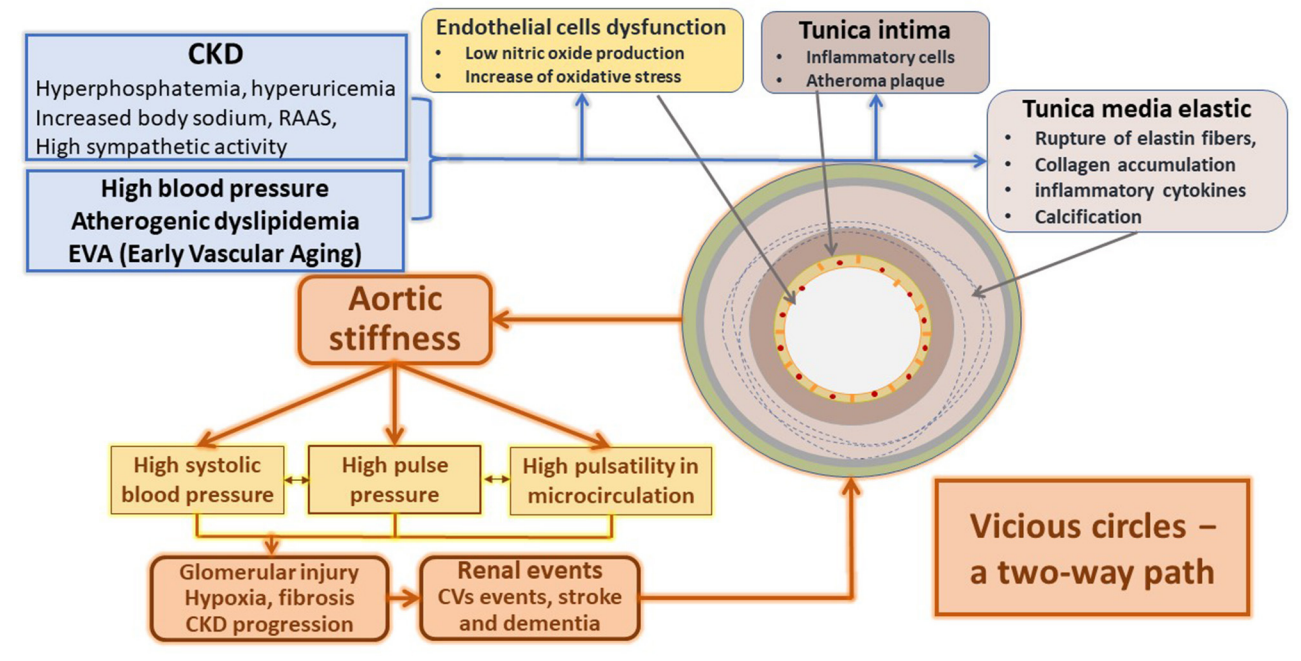


FIGURE 2 | Main mechanisms responsible for the structural and functional changes of the arteries in CKD.

independently associated with mortality in CKD patients after adjusting for additional confounders, including inflammation (17).

In other words, the increase in arterial stiffness -represented by the rise in PWV- is usually associated with an increase in the SP with consequent LV overload. In addition, the increase in

PWV and vascular stiffness speeds up the pulse wave's reflection, generating an increase in the aortic augmentation that further increases the CAP, and therefore, left ventricle contractile effort.

So, EVA is a consequence of cardiovascular risk factors (CVRF), amplified by CKD, and this cluster of factors lead to premature cardiovascular events (CVE) (18–20), as well as accelerate the damage of various tissues and their functions, including a faster decline of kidney function.

Association Between Inflammation and Arterial Stiffness

The different tissue lesions associated with CVRF are also related to the activation of diverse inflammatory ways. Initially, they act as a protective response of the organism to control the cause, but finally, they turn into a disease. The inflammatory response to stressors acts on the endothelium and vascular smooth muscle; therefore, serum and tissue inflammatory markers are tools that help to predict cardiovascular disease (CVD) (21–23). **Figure 2** summarizes the primary inflammatory mechanism associated with vascular stiffness in several clinical situations, particularly CKD.

The association between chronic inflammation and arterial wall disease is complicated and multifactorial (24). Initially, the circulation of inflammatory mediators favors leukocytes' migration into the arterial wall (25). Then, macrophages activation by different factors, including metabolic and electrolytic disturbances associated with catecholamines, renin-angiotensin-aldosterone system (RAAS), and endothelin disarrangement associated with cytokines and reactive oxygen species (ROS), amplify the inflammatory reaction.

The subsequent transformation of these macrophages within the arterial wall into foamy cells predisposes to their necrosis; when the necrotic nucleus appears in the plaques, the amplification of the inflammatory stimulus favors the progression of already advanced vascular lesions (26).

This inflammatory cascade also alters the endothelium's function that interacts and conditions the remodeling of the tunica media and changes of the artery's mechanical properties (23). Endothelial cells decrease the usual production of nitric oxide (NO) and increase endothelin (E1), favoring arterial stiffness. In turn, arterial stiffness subsequently alters the endothelium, thus generating a vicious circle (27, 28).

Simultaneously, the increase in arterial stiffness and the dysfunctional endothelium activate adhesion molecules like MCP-1 and cytokines favoring thrombotic events (21, 29, 30). Dendritic cells and T-lymphocytes play an essential role in synthesizing pro-atherogenic cytokines (IL-2, IL-18, and IFN-gamma), responsible for the installation and progression of atherosclerotic plaques (31).

Vascular inflammation enhanced by CKD promotes the vessel's stiffening by stimulating fibrosis and proliferation of the vascular smooth muscle cells (VSMC) (23).

Role of CKD in the Inflammatory State and Vascular Injury

CKD, defined as a structural and functional alteration of the kidney for more than 3 months (32). CKD is a low-grade chronic inflammatory state associated with a significant increase in morbidity and mortality (33). CKD and a set of factors—chronic acidosis, recurrent infections, and altered microbiota—generate increased cytokine production, oxidative stress, and inflammation.

CKD resembles an experimental oxidative stress model which produces severe alterations in many cells (nuclear and mitochondrial DNA deletion, telomeric shortening), tissues, serum, and urinary markers. Oxidative stress is an initial and central contributor to endothelial dysfunction and the inflammatory process, conducting atherosclerotic vascular injury, premature aging, and CVD (34). A decrease in anti-aging defenses (like Klotho and Fetuin-A activity) increases pro-aging mediators such as angiotensin II, aldosterone, and phosphate, generating a clear discrepancy between chronological and vascular biological age (35, 36).

Inflammation that accompanies kidney disease seems to play a significant role in telomeric shortening and mitochondrial dysfunction (37). Additionally, Galvan et al. have shown a low number of mitochondria, also dysfunctional, in most of the tissues of patients with CKD, representing a primary metabolic-energetic alteration present in these patients from very early stages (38). It is an essential component of the disease and the primary source of increased reactive oxygen species production. This inflammatory state also decreases the body's resistance to external stressors, thus conditioning a state of increased vulnerability (33).

At the same time, the kidney itself is vulnerable to this inflammatory process. The kidneys are intensely and heterogeneously vascularized and regulated by hormones and vasoactive molecules (like RAAS, prostaglandins, endothelin, NO, and others) (39, 40). Systemic inflammation favors the intrarenal inflammatory cascade associated with tubular and glomerular injury and, therefore, generation and progression of CKD.

Systemic inflammation eases the development of renal injury and is co-responsible for the high morbidity and mortality of these patients and the development of an accelerated aging phenotype.

In CKD, calcification of the middle layer of the arteries is a part of the accelerated EVA process. Therefore, the extent of vascular calcium vascular deposits is related to vascular estimated age. That is why in CKD, the age of the vasculature is practically always older than the chronological age, at least partially, due to an early and persistent inflammatory process.

Unlike what happens in individuals with preserved kidney function, those with CKD have a process of accelerated cellular and vascular senescence, tissue aging, persistent inflammation, loss of muscle mass, osteoporosis, and early general fragility.

In other words, EVA in patients with CKD is part of the price paid for the enormous allostatic load, a consequence of multiple physical and inflammatory stressors associated with CKD.

The Partial Reversion of Vascular Changes by Renal Transplantation

It is to point out that recovering kidney function with a kidney transplant (Tx) decreases the mortality rate by 50% compared to the same patient population submitted to dialysis treatment.

Hence, it raises whether a kidney transplant may reduce the described vascular changes suffered along with CKD.

Few studies have shown after transplant regression of the large arteries remodeling; however, there is evidence about the decrease of the PWV after Tx, which increases the patient and the graft survival rate (41, 42).

The French Group Karras published the data of 161 consecutive Tx patients. The outcome was the arterial parameters, measured at 3 and 12 months after kidney transplant. The results showed that mean PWV decreases from 10.8 m/s in the 3rd month to 10.1 m/s after 12 months ($p < 0.001$). After the multivariate analysis, the patients who received Tx living donor allograft had a more significant decrease of the PWV ($p < 0.001$). Furthermore, the patients who received deceased allograft with standard donors had better vascular performance than those who received allograft from donors with expanded criteria (older donors and pre-establish cardiovascular disease). An interesting point to highlight in this study is the non-relationship between vascular function improvement and glomerular filtration level (43). The progression of arterial stiffness after 12 months of kidney Tx was also studied in 28 patients, as a control group was studied, 23 hemodialysis patients. The decrease in the PWV, measured with SphygmoCor, in Tx patients was higher than patients under hemodialysis treatment ($p < 0.0001$) (44).

Korogiannou et al. recently confirmed the relevance of PWV in the prognosis of Tx patients been a strong predictor for cardiovascular events, renal events, and mortality in these individuals (45).

Although scientific facts are clear enough, there are still two significant aspects to consider. First, there are no clinical trials about the impact of therapeutic interventions on arterial stiffness and its consequence on the kidney Tx population. Also, mechanistic studies are required to identify the best ways to address arterial stiffness in Tx patients.

UREMIC TOXINS AS VASCULAR TOXINS

Hyperphosphatemia and its consequences: During CKD, an imbalance between the inhibitors and inducers of vascular calcification occurs (46).

The decrease in renal phosphate excretion increases serum levels and promotes the calcification process by activating the Toll-like receptor four and NF-Kappa B in VSMC (47). Also, in the context of hyperphosphatemia, VSMC changes its phenotype to osteoblastic-like cells *via* the expression of ossifying genes (48). Likewise, phosphates also produce mitochondrial dysfunction, with increased reactive oxygen species production, activation of pro-inflammatory molecules, and increased tumor necrosis factor (TNF).

CKD also alters hormonal processes that regulate phosphate levels (Intestinal absorption, renal excretion by remaining nephrons, bone metabolism modulated by vitamin D, fetuin-A, Klotho, and fibroblast growth factor 23 (FGF-23) (49). Calcium deposits concentrated in the tunica media and the vascular wall's subendothelium are an essential part of the problem. A detailed description of the facts exceeds this manuscript's objectives.

Uric acid increases in CKD due to the decrease excretion by the failing kidney. This mentioned uric acid elevation decreases endothelial Nitric Oxide Synthase (eNOS) activity, reducing the production of NO, the proliferation of VSMC (50, 51), the expression of COX-2, and the increase in the production of angiotensin II, contributing to arterial stiffness.

Advanced glycation ends products (AGEs): AGEs accumulate in CKD progressively as their production increases and elimination decreases. Thus, significant accumulation may occur even in non-diabetic patients. AGEs, among other things, affect the activity of eNOS (52), favor the phenotypic change of the VSMC, and the "cross-linking" of collagen (the changes of its composition make the arterial wall less compliant). They also activate NF Kappa B, favoring the activation of the vascular inflammatory cascade and structural stiffening.

The same happens with the increase in asymmetric dimethylarginine (ADMA) resulting from increased production and less excretion, contributing to a significant reduction of eNOS and consequent endothelial dysfunction. Increased ADMA also causes sympathetic stimulation, inflammation, vascular stiffness, and LVH (53–56).

Increment of endothelin-1 (E-1) level: E-1 is a potent vasoconstrictor implicated in cardiovascular and renal diseases. An increase of E-1 has the same origin as the increase of ADMA and uric acid. It acts on receptors with antagonistic function (ET_A and ET_B receptors), predominantly the action of ETA receptor, which is responsible for: endothelial dysfunction, increased vascular tone, inflammation, and calcification (57).

Renal ET-1 production increases associated with CKD progression, and a cluster of conditions frequently present in these patients, such as diabetes, insulin resistance, obesity, immune system activation, atherogenic dyslipidemia, nitric oxide deficiency, and oxidative stress (58).

Vascular Inflammation in Dialysis Patients

The dialysis procedure generates additional inflammation that adds to those already described and known in CKD. All inflammatory cytokines are markedly high in dialysis patients (IL-1, IL-6, IL-23, and TNF alfa), as well as high-sensitivity C-reactive protein and fibrinogen. Albumin, as an acute-phase reactant, is decreased (59).

Frequent infections, thrombotic events, dialysate quality, and its impurities are also powerful inflammatory stimuli. Uremia increases intestinal permeability to bacteria, and this, in turn, generates more inflammation. The diets indicated in these patients (Low in potassium and phosphorous) alter the microbiota, causing dysbiosis with significant inflammatory effects. Dialysis patients usually have markedly high inflammatory markers associated with severe arterial injuries that, in turn, progress faster (60).

ARTERIAL STIFFNESS AND THE BARORREFLEX FUNCTION

The baroreflex system regulates blood pressure changes, and their proper function enables immediate regulation of it at

practically constant values. Its appropriate process depends mainly on arterial compliance. Vascular lesions are widespread at the carotid and aortic levels and affect the baroreflex system's receptors. Receptors activation requires good arterial compliance; potassium channels and sodium-potassium pump regulated by a paracrine function, mainly by prostacyclin.

The combination of endothelial dysfunction and arterial stiffness produces a decrease in prostacyclin production and less arterial compliance. Consequently, less baroreflex activation occurs, causing more significant variability in arterial pressure (i.e., greater blood pressure drop when standing up). Simultaneously, there is a greater renal afference toward the central nervous system in CKD. An increase in the sympathetic activity, a further increase in the vascular tone favors LVH, CVD, and increased mortality (61). The high prevalence and severity of baroreflex dysfunction in CKD patients were recently reviewed, and how afferent and efferent pathways between kidney and brain may deteriorate its function (62, 63).

From Arterial Stiffness to Myocardial Dysfunction

Arterial stiffness and CKD volume overload generate myocardial dysfunction directly proportional to the degree of renal failure (64).

This myocardial involvement resulting from increased pre and afterload is also associated with cardiac interstitial fibrosis, alteration of the cardiac microcirculation, and myocardial neurohumoral activation (65).

The most common and earlier stage of ventricular dysfunction in CKD patients is diastolic failure. It is also known as HF with preserved ventricular function, the usual echocardiographic finding in CKD patients (66).

These patients frequently have other risk factors for diastolic failures, such as type 2 diabetes, high blood pressure, coronary heart disease, and accelerated aging, all contributing to maintaining and worsening diastolic dysfunction.

The structural changes of the heart in CKD include myocardial hypertrophy and thickening of the intramural arteries (67) as an adaptative response to changes in volume and pressure. Finally, what was initially an adaptative response, leads to myocardial fibrosis due to all the metabolic and neurohumoral disorders previously described.

Other CKD alterations that further aggravate myocardial dysfunction are the over activation of systemic and intrarenal RAAS, the anemia that characterizes patients with CKD, vitamin D deficiency, and other mechanisms recently described, such as activation of mTOR, G-protein activation, and T-cell activation (7); all of them may influence cardiac structure and function. In addition, the synergy of all these factors activates apoptosis and autophagy pathways, which increase the production of extracellular matrix in the myocardium, and lead to decreased left ventricular compliance since fibrotic tissue predominates over the cardiac muscle.

The clinical consequence of all these processes is a shift to the left of the pressure-volume curve. Small changes in volume significantly increase intraventricular pressure due to cardiac compliance loss and can cause pulmonary congestion. Conversely, slight volume depletion can impair left ventricular filling and cause a decrease in systolic volume, leading to hypotension and hemodynamic instability (66).

In other words, patients with CKD have a low range of tolerance to volume changes, extrapolated to body weight, to go from volume overload to hypotension, generating an increase in hospitalizations for decompensated heart failure. In addition, in some patients, particularly the young, functional damage to the left ventricle due to volume overload may not be evident, but it will deteriorate cardiac function if overhydration persists (68).

In addition to the CKD-dependent changes in vascular structure and function already described, we must add those that depend on the diseases frequently associated. Those most common are coronary artery disease, vascular injuries, and remodeling that depends on high blood pressure, atherogenic dyslipidemia, diabetes, and their associated metabolic disorders, together with accelerated vascular aging to these pathologies.

The detailed description of these processes exceeds the objective of this publication. Several reviews (69–75) are available that deal extensively with these factors' influence on vascular changes and cardiac disease. **Figure 2** shows a comprehensive synthesis integrating the main mechanisms that generate the systemic vasculature alterations in CKD.

THE OTHER WAY: FROM VASCULAR INJURY TO CHRONIC KIDNEY DISEASE

Under a healthy vascular condition, the large arteries' elasticity moderates the cardiac pulse pressure, dampening its intensity, achieving a continuous flow of blood, in most tissues, with a low variation in arterial pressure between systole and diastole. That means a low pulse pressure. This low arterial pulsatility enters from the macrocirculation to the microcirculation, where it receives additional attenuation in the arterioles and results in microvasculature protection. However, in response to aging (76), obesity, diabetes mellitus, and mainly CKD, an increased arterial stiffening reduce the central arteries' buffering capacity, generating high pulsatile stress at the microvasculature level.

As a result, the high pulsatility introduced into the organs; of particular interest are those tissues with high viscous components such as the brain and the kidneys; both are characterized by low resistance and high flow systems, thus receiving a high volume of blood (77, 78). The main consequences of these events will be functional deterioration with the development or acceleration of cognitive disorders and renal function impairment (79). Considering that high pulsatility causes damage to the microvasculature, strategies to reduce it could slow the progression of kidney disease and associated events (80).

Several renal diseases that reduce renal mass generate an adaptive high filtration rate by a single nephron. This process also happens in diabetes, obesity, hypertension, and aging. These clinical conditions are of great interest due to their high frequency.

Glomerular hyperfiltration (GHF) or single nephron hyperfiltration is an increased glomerular filtration rate above

normal values due to increased filtration per nephron unit. There is robust evidence that GHF is a risk factor for the progression of chronic kidney disease and CV events, independently of albuminuria and other factors. Remarkably, in patients with GHF, an increase in PP was proved, measured during 24-h, suggesting an association with increased large-artery stiffness and vascular damage, leading to increased CV events (81, 82).

GHF can occur in individuals with high, normal, and low GFR, as happens in most CKD patients moderate/severe stages (83). In CKD patients with low GFR, the diagnosis of GHF in the clinical practice is very challenging, but the association exists in most patients. We had previously mentioned the usual association of GHF with arterial stiffness and high PP and pulsatility.

Pieces of evidence generated more than two decades ago confirmed that pulsatile hypertension-induced glomerular distention produces changes at a cellular level and the extracellular matrix's metabolism. The changes due to mesangial cell mechanical strain occur in the remnant kidney and play an essential pathogenetic role in renal lesions. These changes were initially described in experimental diabetes and renal failure by glomerular mass reduction as the experimental model of 5/6 nephrectomy (84, 85).

Of interest, renal blood flow increases together with dilation of the afferent arteriole in the enlarged glomeruli of both models, animals with diabetes, and subtotal nephrectomies. Therefore, systemic blood pressure is transmitted into the glomerulus without the usual regulation, generating a high pulsatile stretching in the glomerular and surrounding structures. This persistent pulsatile stretch, in turn, changes the phenotypes of mesangial cells that increase the production of different cytokines producing the recruitment of cells leading to inflammation and kidney fibrosis (86, 87).

More recently, using Doppler devices, it is possible to evaluate renal microvascular pulsatility. The pulsatility index derived from pulsed-wave Doppler measurements correlates with effective renal plasma flow in CKD patients and predicts renal disease progression (88, 89).

These pathophysiological mechanisms described before are in line with consistent epidemiological results that show the association between arterial stiffness, microcirculation pulsatility, and the incidence and progression of renal diseases, as well as hard renal endpoints (90, 91).

Reducing or controlling GHF and restoring to normal the disturbed glomerular hemodynamics has been the most crucial strategy for glomerular protection and to slow the progression of chronic kidney disease. Paradigmatic drugs in kidney protection, such as those that block the renin-angiotensin system (RAS inhibitors), or the new ones such as sodium-glucose cotransport type 2 inhibitors (iSGLT2), and the GLP-1 receptor agonists (GLP-1), produces, by different mechanisms, a consistent reduction of GHF (82).

In summary, a complex variety of mechanisms leading to the damage of arteries in CKD patients, generating stiffness in the aorta and central arteries and increased PP and CAP. Additionally, a higher central pulsatility is transmitted into the microcirculation of various tissues, including kidneys, favoring and accelerating its deterioration. A better knowledge of these pathways and processes leading to this vicious circle of the two-way path between arterial stiffness and renal dysfunction will give the medical community better possibilities to improve preventive and therapeutic strategies to reduce vascular injuries and CKD progression, and finally, cardiovascular events.

AUTHOR CONTRIBUTIONS

FI, PF, and CC conceived of the presented idea. AC wrote the paper with input from the other authors. All the authors reviewed the final version of the manuscript.

REFERENCES

1. Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, et al. Chronic kidney disease and mortality risk: a systematic review. *J Am Soc Nephrol.* (2006) 17:2034–47. doi: 10.1681/ASN.2005101085
2. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet.* (2010) 375:2073–81. doi: 10.1016/S0140-6736(10)60674-5
3. Weir MR. Recognizing the link between chronic kidney disease and cardiovascular disease. *Am J Manag Care.* (2011) 17(Suppl.15):S396–402.
4. Jankowski J, Floege J, Fliser D, Böhm M, Marx N. Cardiovascular disease in chronic kidney disease: pathophysiological insights and therapeutic options. *Circulation.* (2021) 143:1157–72. doi: 10.1161/CIRCULATIONAHA.120.050686
5. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubiña P, Lahera V, Luño J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. *Kidney Int*. (2008) (Suppl.111):S4–59. doi: 10.1038/ki.2008.516
6. Ronco C, Haapio M, House AA, Anavekar N, Bellomo R. Cardiorenal syndrome. *J Am Coll Cardiol.* (2008) 52:1527–39. doi: 10.1016/j.jacc.2008.07.051
7. Zoccali C, Goldsmith D, Agarwal R, Blankestijn PJ, Fliser D, Wiecek A, et al. The complexity of the cardio-renal link: taxonomy, syndromes, and diseases. *Kidney Int Suppl.* (2011) 1:2–5. doi: 10.1038/kisup.2011.4
8. Zanoli L, Empana JP, Perier MC, Alivon M, Ketthab H, Castellino P, et al. Increased carotid stiffness and remodeling at early stages of chronic kidney disease. *J Hypertens.* (2019) 2019:2007. doi: 10.1097/HJH.0000000000002007
9. Guerin AP, Blacher J, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation.* (2001) 103:987–92. doi: 10.1161/01.CIR.103.7.987
10. Townsend RR. Arterial stiffness in CKD: a review. *Am J Kidney Dis.* (2019) 73:240–7. doi: 10.1053/j.ajkd.2018.04.005
11. Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries*. 4th ed. London: Arnold (1998).
12. O'Rourke MF. *Arterial Function in Health and Disease*. Edinburgh: Churchill Livingstone (1982).
13. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. *Circulation.* (1999) 99:2434–45. doi: 10.1161/01.CIR.99.18.2434
14. London G, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial wave re?ections and survival in end-stage renal failure. *Hypertension.* (2001) 38:434–9. doi: 10.1161/01.HYP.38.3.434
15. Towsend R. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. *Curr*

Opin Nephrol. (2015) 24:47–53. doi: 10.1097/MNH.0000000000000086
16. Townsend RR, Anderson AH, Chirinos JA, Feldman HI, Grunwald JE, Nessel L, et al. Association of pulse wave velocity with chronic kidney disease progression and mortality: findings from the CRIC study (Chronic Renal Insufficiency Cohort). *Hypertension.* (2018) 71:1101–7. doi: 10.1161/HYPERTENSIONAHA.117.10648
17. Mukai H, Svedberg O, Lindholm B, Dai L, Heimbürger O, Barany P, et al. Skin autofluorescence, arterial stiffness and Framingham risk score as predictors of clinical outcome in chronic kidney disease patients: a cohort study. *Nephrol Dial Transplant.* (2019) 34:442–8. doi: 10.1093/ndt/gfx371
18. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol.* (2005) 25:932–43. doi: 10.1161/01.ATV.0000160548.78317.29
19. Adel M, ELSheikh A, Sameer S, Haseeb W, ELSheikh E, Kheder L. Arterial stiffness in metabolic syndrome. *J Saudi Heart Assoc.* (2016) 28:249–56. doi: 10.1016/j.jsha.2015.12.005
20. Mozos I, Stoian D, Luca CT. Crosstalk between vitamins A, B12, D, K, C and E status and arterial stiffness. *Dis Markers.* (2017) 2017:8784971. doi: 10.1155/2017/8784971
21. Vita JA, Keaney JF Jr, Larson MG, Keyes MJ, Massaro JM, Lipinska I, et al. Brachial artery vasodilator function and systemic inflammation in the Framingham Offspring Study. *Circulation.* (2004) 110:3604–9. doi: 10.1161/01.CIR.0000148821.97162.5E
22. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation.* (2002) 105:1135–43. doi: 10.1161/hc0902.104353
23. Drechsler M, Megens RTA, Van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophylia promotes early atherosclerosis. *Circulation.* (2010) 122:1837–45. doi: 10.1161/CIRCULATIONAHA.110.961714
24. Aznaouridis KA, Stefanadis CI. Inflammation and arterial function. *Artery Res.* (2007) 1:32–8. doi: 10.1016/j.artres.2007.03.005
25. Yildiz M. Arterial distensibility in chronic inflammatory rheumatic disorders. *Open Cardiovasc Med J.* (2010) 4:83–8. doi: 10.2174/1874192401004010083
26. Jones DP, True HD, Patel J. Leukocyte trafficking in cardiovascular disease: insights from experimental models. *Mediators Inflamm.* (2017) 2017:9746169. doi: 10.1155/2017/9746169
27. Shirwany NA, Zou M. Arterial stiffness: a brief review. *Acta Pharmacol Sin.* (2010) 31:1267–76. doi: 10.1038/aps.2010.123
28. Avolio A, Butlin M, Liu YY, Viegas K, Avadhanam B, Lindesay G. Regulation of arterial stiffness: cellular, molecular and neurogenic mechanisms. *Artery Res.* (2011) 5:122–7. doi: 10.1016/j.artres.2011.10.002
29. Lacolley P, Challande P, Regnault V, Lakatta EG, Wang M. Cellular and molecular determinants of arterial aging. In: Nilsson P, Olsen M, Laurent S, editors, *Early Vascular Aging*. Cambridge, MA; Amsterdam: Academic Press; Elsevier (2015). p. 7–14. doi: 10.1016/B978-0-12-801387-8.00002-8
30. Smulyan H, Mookherje S, Safar ME. Two faces of hypertension: role of aortic stiffness. *J Am Soc Hypertens.* (2016) 10:175–83. doi: 10.1016/j.jash.2015.11.012
31. Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol.* (2011) 12:204–12. doi: 10.1038/ni.2001
32. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. *Kidney Int.* (2017) 92:26–36. doi: 10.1016/j.kint.2017.04.006
33. Go AS. Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risk of dead, cardiovascular events and hospitalization. *NEJM.* (2004) 351:1296–305. doi: 10.1056/NEJMoa041031
34. Krata N, Zagozdzon R, Foroncewicz B, Mucha K. Oxidative stress in kidney diseases: the cause or the consequence? *Arch Immunol Ther Exp.* (2018) 66:211–20. doi: 10.1007/s00005-017-0496-0
35. Dai L, Qureshi AR, Witasp A, Lindholm B, Stenvinkel P. Early vascular ageing and cellular senescence in chronic kidney disease. *Comput Struct Biotechnol J.* (2019) 17:721–9. doi: 10.1016/j.csbj.2019.06.015
36. Hobson S, Arefin S, Kublickiene K, Shiels PG, Stenvinkel P. Senescent cells in early vascular ageing and bone disease of chronic kidney disease—a novel target for treatment. *Toxins.* (2019) 11:E82. doi: 10.3390/toxins11020082
37. Kooman JP, Dekker MJ, Usvyat LA, Kotanko P, van der Sande FM, Schalkwijk CG, et al. In?ammation and premature aging in advanced chronic kidney disease. *Am J Physiol Renal Physiol.* (2017) 313:F938–50. doi: 10.1152/ajprenal.00256.2017
38. Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. *Kidney Int.* (2017) 92:1051–7. doi: 10.1016/j.kint.2017.05.034
39. Akchurin OM, Kaskel F. Update on inflammation in chronic kidney disease. *Blood Purificat.* (2015) 39:84–92. doi: 10.1159/000368940
40. Damkjær M, Vafaee M, Møller ML, Braad PE, Petersen H, Høilund-Carlsen PF, et al. Renal cortical and medullary blood flow responses to altered NO availability in humans. *Innov Method.* (2010) 299:R1449–55. doi: 10.1152/ajpregu.00440.2010
41. Zoungas S, Kerr PG, Chadban S, Muske C, Ristevski S, Atkins RC, et al. Arterial function after successful renal transplantation. *Kidney Int.* (2004) 65:1882–9. doi: 10.1111/j.1523-1755.2004.00595.x
42. Covic A, Goldsmith DJ, Gusbeth-Tatomir P, Buhaescu I, Covic M. Successful renal transplantation decreases aortic stiffness and increases vascular reactivity in dialysis patients. *Transplantation.* (2003) 76:1573–7. doi: 10.1097/01.TP.0000086343.32903.A8
43. Karras A, Boutouyrie P, Briet M, Bozec E. Reversal of arterial stiffness and maladaptative arterial remodeling after kidney transplantation. *J Am Heart Assoc.* (2017) 6:e006078. doi: 10.1161/JAHA.117.006078
44. Keven K, Calayoglu R, Sengul S, Dincer I, Kutlay S, Erturk S, et al. Comparative effects of renal transplantation and maintenance dialysis on arterial stiffness and left ventricular mass index. *Clin Transplant.* (2008) 22:360–5. doi: 10.1111/j.1399-0012.2008.00794.x
45. Korogiannou M, Xagas E, Marinaki S, Sarafidis P, Boletis JN. Arterial stiffness in patients with renal transplantation; associations with co-morbid conditions, evolution, and prognostic importance for cardiovascular and renal outcomes. *Front Cardiovasc Med.* (2019) 6:67. doi: 10.3389/fcvm.2019.00067
46. Vervloet M, Cozzolino M. Vascular calcification in chronic kidney disease: different bricks in the wall? *Kidney Int.* (2017) 91:808–17. doi: 10.1016/j.kint.2016.09.024
47. Zhang D, Bi X, Liu Y, Huang Y, Xiong J, Xu X, et al. High phosphate-induced calcification of vascular smooth muscle cells is associated with the TLR4/NF-kb signaling pathway. *Kidney Blood Press Res.* (2017) 42:1205–15. doi: 10.1159/000485874
48. Lacolley P, Regnault V, Segers P, Laurent S. Vascular smooth muscle cells and arterial stiffening: Relevance in development, aging, and disease. *Physiol Rev.* (2017) 97:1555–617. doi: 10.1152/physrev.00003.2017
49. Kuro-O M. Phosphate as a pathogen of arteriosclerosis and aging. *J Atheroscler Thromb.* (2021) 28:203–13. doi: 10.5551/jat.RV17045
50. Park JH, Jin YM, Hwang S, Cho DH, Kang DH, Jo I. Uric acid attenuates nitric oxide production by decreasing the interaction between endothelial nitric oxide synthase and calmodulin in human umbilical vein endothelial cells: a mechanism for uric acid-induced cardiovascular disease development. *Nitric Oxide.* (2013) 2832:36–42. doi: 10.1016/j.niox.2013.04.003
51. Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am J Physiol Renal Physiol.* (2002) 282:F991–7. doi: 10.1152/ajprenal.00283.2001
52. Jamwal S, Sharma S. Vascular endothelium dysfunction: a conservative target in metabolic disorders. *In?amm Res.* (2018) 67:391–405. doi: 10.1007/s00011-018-1129-8
53. Tripepi G, Mattace Raso F, Sijbrands E, Seck MS, Maas R, Boger R, et al. In?ammation and asymmetric dimethylarginine for predicting death and cardiovascular events in ESRD patients. *Clin J Am Soc Nephrol.* (2011) 6:1714–21. doi: 10.2215/CJN.11291210
54. Zoccali C, Mallamaci F, Maas R, Benedetto FA, Tripepi G, Malatino LS, et al. CREED Investigators: left ventricular hypertrophy, cardiac remodeling and asymmetric dimethylarginine (ADMA) in hemodialysis patients. *Kidney Int.* (2002) 62:339–45. doi: 10.1046/j.1523-1755.2002.00437.x
55. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, et al. Asymmetric dimethylarginine, C-reactive protein, and carotid intimamedia thickness in end-stage renal disease. *J Am Soc Nephrol.* (2002) 13:490–6. doi: 10.1681/ASN.V132490

56. Kielstein JT, Donnerstag F, Gasper S, Menne J, Kielstein A, Martens-Lobenhoffer J, et al. ADMA increases arterial stiffness and decreases cerebral blood flow in humans. *Stroke.* (2006) 37:2024–9. doi: 10.1161/01.STR.0000231640.32543.11
57. Dhaun N, Goddard J, Webb DJ. The endothelin system and its antagonism in chronic kidney disease. *J Am Soc Nephrol.* (2006) 17:943–55. doi: 10.1681/ASN.2005121256
58. Barton M. Reversal of proteinuric renal disease and the emerging role of endothelin. *Nat Clin Pract Nephrol.* (2008) 4:490–501. doi: 10.1038/ncpneph0891
59. Descamps-Latscha B, Herbelin A, Nguyen AT, Roux-Lombard P, Zingraff J, Moynot A, et al. Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. *J Immunol.* (1995) 154:882–92.
60. Nowak KL, Chonchol M. Does inflammation affect outcomes in dialysis patients? *Semin Dial.* (2018) 31:388–97. doi: 10.1111/sdi.12686
61. Gupta A, Jain G, Kaur M, Jaryal AK, Deepak KK, Bhowmik D, et al. Association of impaired baroreflex sensitivity and increased arterial stiffness in peritoneal dialysis patients. *Clin Exp Nephrol.* (2016) 20:302–8. doi: 10.1007/s10157-015-1158-3
62. Underwood CF, Hildreth CM, Wyse BF, Boyd R, Goodchild AK, Phillips JK. Uraemia: an unrecognized driver of central neurohumoral dysfunction in chronic kidney disease? *Acta Physiol.* (2017) 219:305–23. doi: 10.1111/apha.12727
63. Salman IM, Hildreth CM, Ameer OZ, Phillips JK. Differential contribution of afferent and central pathways to the development of baroreflex dysfunction in chronic kidney disease. *Hypertension.* (2014) 63:804–10. doi: 10.1161/HYPERTENSIONAHA.113.02110
64. Franczyk-Skóra B, Gluba A, Olszewski R, Banach M, Rysz J. Heart function disturbances in chronic kidney disease – echocardiographic indices. *Arch Med Sci.* (2014) 10:1109–16. doi: 10.5114/aoms.2014.47822
65. Lopez B, Gonzalez A, Hermida N, Laviades C, Diez J. Myocardial fibrosis in chronic kidney disease: potential benefits of torasemide. *Kidney Int*. (2008) 74(Suppl.111):S19–23. doi: 10.1038/ki.2008.512
66. Glassock RJ, Pecoits-Filho R, Barberato SH. Left ventricular mass in chronic kidney disease and ESRD. *Clin J Am Soc Nephrol*. (2009) 4(Suppl.1):S79–91. doi: 10.2215/CJN.04860709
67. Tyralla K, Amann K. Morphology of the heart and arteries in renal failure. *Kidney Int*. (2003) 63(Suppl.84):S80–3. doi: 10.1046/j.1523-1755.63.s84.1.x
68. Barberato SH, Bucharles SG, Sousa AM, Costantini CO, Costantini CR, Pecoits-Filho R. Prevalence and prognostic impact of diastolic dysfunction in patients with chronic kidney disease on hemodialysis. *Arq Bras Cardiol.* (2010) 94:457–62. doi: 10.1590/S0066-782X2010005000016
69. Briet M, Boutouyrie P, Laurent S, London GM. Arterial stiffness and pulse pressure in CKD and ESRD. *Kidney Int.* (2012) 82:388–400. doi: 10.1038/ki.2012.131
70. Chirinos JA, Segers P, Hughes T, Townsend R. Large-artery stiffness in health and disease: JACC state-of-the-art review. *J Am Coll Cardiol.* (2019) 74:1237–63. doi: 10.1016/j.jacc.2019.07.012
71. Shoji T, Abe T, Matsuo H, Egusa G, Yamasaki Y, Kashihara N, et al. Committee of renal and peripheral arteries, Japan Atherosclerosis Society. Chronic kidney disease, dyslipidemia, and atherosclerosis. *J Atheroscler Thromb.* (2012) 19:299–315. doi: 10.5551/jat.10454
72. Jha JC, Ho F, Dan C, Jandeleit-Dahm K. A causal link between oxidative stress and inflammation in cardiovascular and renal complications of diabetes. *Clin Sci.* (2018) 132:1811–36. doi: 10.1042/CS20171459
73. Bangalore S, Fayyad R, Hovingh GK, Laskey R, Vogt L, DeMicco DA, et al. Treating; to new targets steering committee and investigators. *Am J Cardiol.* (2014) 113:2018–20. doi: 10.1016/j.amjcard.2014.03.046
74. Gajdova J, Karasek D, Goldmannova D, Krystynik O, Schovanek J, Vaverkova H, et al. Pulse wave analysis and diabetes mellitus. A systematic review. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* (2017) 161:223–33. doi: 10.5507/bp.2017.028
75. Maloberti A, Vallerio P, Triglione N, Occhi L, Panzeri F, Bassi I, et al. Vascular aging and disease of the large vessels: role of inflammation. *High Blood Press Cardiovasc Prev.* (2019) 26:175–82. doi: 10.1007/s40292-019-00318-4
76. McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol.* (2005) 46:1753–60. doi: 10.1016/j.jacc.2005.07.037
77. Laurent S, Boutouyrie P. The structural factor of hypertension. *Circ Res.* (2015) 116:1007–21. doi: 10.1161/CIRCRESAHA.116.303596
78. Climie RE, Picone DS, Blackwood S, Keel SE, Qasem A, Rattigan S, et al. Pulsatile interaction between the macro-vasculature and micro-vasculature: proof-of-concept among patients with type 2 diabetes. *Eur J Appl Physiol.* (2018) 118:2455. doi: 10.1007/s00421-018-3972-2
79. Hughes TM, Craft S, Lopez OL. Review of the potential role of arterial stiffness in the pathogenesis of Alzheimer's disease. *Neurodegener Dis Manag.* (2015) 5:121–35. doi: 10.2217/nmt.14.53
80. Climie RE, Gallo A, Picone DS, Di Lasico N, van Sloten TT, Guala A, et al. Measuring the interaction between the macro- and micro-vasculature. *Front Cardiovasc Med.* (2019) 6:169. doi: 10.3389/fcvm.2019.00169
81. Reboldi G, Verdecchia P, Fiorucci G, Beilin LJ, Eguchi K, Imai Y, et al. Glomerular hyperfiltration is a predictor of adverse cardiovascular outcomes. *Kidney Int.* (2018) 93:195–203. doi: 10.1016/j.kint.2017.07.013
82. Kanbay M, Ertuglu LA, Afsar B, Ozdogan E, Kucuksumer ZS, Ortiz A, et al. A risk factor for cardiovascular disease and mortality. *Diabetes Obes Metab.* (2019) 21:2368–83. doi: 10.1111/dom.13831
83. Zoccali C, Mallamaci F. The overdriven glomerulus as a cardiovascular risk factor. *Kidney Int.* (2018) 93:13–5. doi: 10.1016/j.kint.2017.08.035
84. Cortes P, Zhao X, Riser BL, Narins RG. Role of glomerular mechanical strain in the pathogenesis of diabetic nephropathy. *Kidney Int*. (1997) 51:57–68. doi: 10.1038/ki.1997.8
85. Cortes P, Zhao X, Riser BL, Narins RG. Regulation of glomerular volume in normal and partially nephrectomized rats. *Am J Physiol.* (1996) 270:F356–370. doi: 10.1152/ajprenal.1996.270.2.F356
86. Riser BL, Cortes P, Zhao A, Bernstein J, Dumler F, Narins RG. Intraglomerular pressure and mesangial stretching stimulate extracellular matrix formation in the rat. *J Clin Invest.* (1992) 90:1932–43. doi: 10.1172/JCI116071
87. Riser BL, Cortes P, Heilig C, Grondin J, Ladson-Wofford S, Patterson D, et al. Cyclic stretching force selectively up-regulates transforming growth factor-f3 isoforms in cultured rat mesangial cells. *Am J Pathol.* (1996) 148:1915–23.
88. Petersen LJ, Petersen JR, Talleruphuus U, Ladefoged SD, Mehlsen J, Jensen HA. The pulsatility index and the resistive index in renal arteries. Associations with long-term progression in chronic renal failure. *Nephrol Dial Transplant.* (1997) 12:1376–80. doi: 10.1093/ndt/12.7.1376
89. Cicoira M, Conte L, Rossi A, Bonapace S, D'Agostini G, Dugo C, et al. Renal arterial pulsatility predicts progression of chronic kidney disease in chronic heart failure patients. *Int J Cardiol.* (2013) 167:3050–1. doi: 10.1016/j.ijcard.2012.11.088
90. Georgianos PI, Sarafidis PA, Liakopoulos V. Arterial stiffness: a novel risk factor for kidney injury progression? *Am J Hypertens.* (2015) 28:958–65. doi: 10.1093/ajh/hpv004
91. Michener KH, Mitchell GF, Noubary F, Huang N, Harris T, Andresdottir MB, et al. Aortic stiffness and kidney disease in an elderly population. *Am J Nephrol.* (2015) 41:320–8. doi: 10.1159/000431332

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 25 November 2021
doi: 10.3389/fmed.2021.769335

The Glomerular Filtration Rate: From the Diagnosis of Kidney Function to a Public Health Tool

Ana Maria Cusumano[1]*, *Carmen Tzanno-Martins*[2] *and Guillermo Javier Rosa-Diez*[3]

[1] Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Buenos Aires, Argentina, [2] Independent Researcher, São Paulo, Brazil, [3] Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

The prevalence of chronic kidney disease (CKD) continues to increase worldwide, as well as the associated morbidity and mortality and the consequences on the patients' quality of life and countries' economies. CKD often evolves without being recognized by patients and physicians, although the diagnosis is based on two simple laboratory data: the estimated glomerular filtration rate (eGFR) and urine analysis. To measure GFR, the knowledge about the physiologic processes at the nephron level, the concept of clearance, and the identification of creatinine as a suitable endogenous marker for measuring the creatinine clearance (CrCl) had to be previously developed. On those bases, different equations to calculate CrCl (Cockcroft and Gault, 1976), or estimated GFR (four variables MDRD, 1999; CKD-Epi, 2009, among others) were generated. They all include creatinine and some demographic data, such as sex and age. However, to compare results throughout life or among laboratories, the creatinine determination must be standardized. In addition, the accuracy of these equations remains controversial in certain subgroups of patients. For these reasons, other mathematical models to improve CrCl estimation have been developed, such as when urine cannot be collected, in debilitated elderly patients and patients with trauma, diabetes, or obesity. Currently, eGFR in adults can be measured and reported immediately, using isotope dilution mass spectrometry traceable creatinine-based equations. In conclusion, based on knowledge obtained from renal physiology, eGFR can be used in the clinic for the diagnosis and early treatment of CKD, as well as a public instrument to estimate the prevalence.

Keywords: glomerular filtration rate, chronic kidney disease, MDRD study equation, CKD-EPI equation, cystatin C, creatinine clearance

OPEN ACCESS

Edited by:
Sergey Brodsky,
Ohio State University Hospital,
United States

Reviewed by:
Sebastjan Bevc,
Maribor University Medical Centre, Slovenia
Lena Jafri,
Aga Khan University, Pakistan

***Correspondence:**
Ana Maria Cusumano
anacusumano@yahoo.com.ar

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *01 September 2021*
Accepted: *25 October 2021*
Published: *25 November 2021*

Citation:
Cusumano AM, Tzanno-Martins C and Rosa-Diez GJ (2021) The Glomerular Filtration Rate: From the Diagnosis of Kidney Function to a Public Health Tool. Front. Med. 8:769335. doi: 10.3389/fmed.2021.769335

INTRODUCTION

The prevalence of chronic kidney disease (CKD) continues to increase worldwide, as well as the associated morbidity and mortality and the consequences on patients' quality of life and countries' economies. In the year 2018, a joint document of the ASN, ERA-EDTA, and ISN societies estimated that over 850 million people worldwide (11% of the total population) lived with kidney disease, about twice the number of diabetic patients estimated by the IDF (422 million) (1–3). An analysis of the Global Burden of Disease Study stated that CKD as cause of death rose from position 25 in the year 1990 to the 17th in 2015 (4). Another publication from the same study estimated that CKD as a mortality cause would ascend to the 5th place by 2040 (5). Besides, CKD is an independent risk factor for cardiovascular disease, and a risk multiplier in other non-communicable chronic

diseases such as hypertension, diabetes, and cardiovascular (6). These data confirm that CKD is a major public health problem.

Any clinical situation resulting from a reduction in the number of functioning nephrons can evolve to CKD, defined by KDIGO guidelines as "abnormalities in kidney structure or function, present for 3 months, with implications for health." The same guideline classifies CKD based on the cause, glomerular filtration rate (GFR) category, and albuminuria (7).

Arterial hypertension, diabetes, obesity, proteinuric nephropathies, race, family history, genetic diseases, low birth weight, aging, among others, are risk factors for the CKD (8, 9). Early detection and treatment of potentially reversible risk factors and CKD allow to delay progression and its associated complications as well as reduce the risk of cardiovascular disease (10, 11).

In the real world, CKD is a silent disease that often evolves unrecognized by the patients and physicians, although the diagnosis is based on the two simple laboratory data: the estimated GFR (eGFR) and urine analysis (screening for albuminuria/proteinuria) (10). Early diagnosis of CKD by the general practitioners and generalists would contribute to retard progression, and reduce morbidity and mortality associated to CKD and its associated risk factors (12).

Glomerular filtration rate continues to be the best global index of kidney function, both in health and in disease, as it represents the excretory capacity of the kidney, correlates directly with the kidney functioning mass, to classify CKD in stages according to the risk of progression, and to calculate the drug dosing and preparing for the invasive studies. xx

Early diagnosis of CKD by the general practitioners and generalists would contribute to retard progression and reduce associated morbidity and mortality. Albuminuria, an important predictor of CKD progression, will not be analyzed in this article. Therefore, the evaluation of kidney function and the presence or absence of proteinuria/albuminuria should be part of any routine health evaluation, and desirable when conducting population health surveys.

The present manuscript, after a brief historical description on the milestones that paved the way since the emerging physiological concepts of filtration, reabsorption, and excretion at the nephron, will focus on the present concepts of eGFR, and how it can be applied in the clinic and as a public health tool. Finally, different eGFR equations derived from creatinine and cystatin C and demographic data used for the diagnosis in patients and as a public health instrument will be described.

The Identification of the Process of Glomerular Filtration to Measuring GFR in the Clinic

Knowledge of kidney physiology began in the mid-19th century, when Carl Ludwig (1816–1895) developed the concept of glomerular filtration. In his thesis, he identified the glomerulus as a filter, where urine formation began; this filter was submitted to physical and chemical forces, driven by the hydrostatic pressure generated by the heart, and regulated by the contraction and vasodilatation of the afferent and efferent arterioles. He went further, speculating that the filtered volume decreased along the tubules due to reabsorption, in order to concentrate the final products at the urine (13, 14).

In 1874, Rudolf Heidenhain (1834–1897) injected a dye, indigo carmine, in hypotensive anuric rabbits; after 15 min he removed the kidneys and identified the dye in tubular cells. He deduced that secretion from blood into the tubule occurred that meant an active tubular transport mechanism (15).

Arthur Cushny (1866–1926) in 1917 reasoned that Ludwig's theory (the glomerulus as a filter) implied a large volume of water, and near all the filtered glucose, amino acids, sodium, and other solutes should be in the ultrafiltrate. And, as these solutes are present at different concentrations in the plasma, the reabsorption of glucose, amino acids, and others dissolved substances present in urine should happen according to their respective blood levels. He concluded that there was a threshold for differential reabsorption of some solutes (16).

In 1924, Alfred Richards (1876–1966) and Joseph Wearns (1893–1984) published their results for the filtration process, infusing epinephrine into the glomerulus of an anesthetized frog, and observing the hemodynamic effects on the afferent and efferent arterioles and the resulting ultrafiltrate. They confirmed that the free-protein ultrafiltrate was due to filtration at the glomerular tuft, the solutes were filtered and reabsorbed at the tubular level, and there was a threshold for glucose reabsorption, corroborating the differential reabsorption of filtered solutes in the tubule (17).

At this point, the mechanism of filtration, secretion, and reabsorption in the nephron had been proved, but to transfer the concept of GFR to the clinic, it was still necessary to find a solute removed only by filtration, and not reabsorbed or secreted in the tubule. In 1926, Paul Rehberg identified creatinine as that solute, as it was produced by the body itself, filtered and, presumably, it was not reabsorbed or excreted (18).

Donald Van Slyke (1883–1971), in 1928, introduced the concept of "clearance," regarding urea, as the volume of blood that would be totally cleared of it in a minute when urine flow exceeded 2 ml/min. The clearance technique was quickly applied to different solutes and became itself a fundamental tool in kidney physiology (19). Applying this concept, in 1937, Homer Smith measured GFR using inulin, a substance he had proved previously was excreted exclusively by glomerular filtration. After that, for many years, inulin was the gold standard to measure true GFR (20).

The clearance concept was fundamental, not only for studying the formation of urine or kidney physiology, but because it provided a simple tool to be used in the clinic, as GFR could be measured as creatinine clearance (CrCl). Since then, over many years, CrCl has been used in the clinic to evaluate GFR.

Creatinine and Creatinine Clearance as Estimated GFR

Creatinine is a waste product of muscle metabolism, generated relatively constantly. It is almost eliminated by the glomerular filtration as it is a small molecule (113 Daltons) not bound to proteins. However, as its concentration depends on muscle mass,

it is different in men and women, and may change according to the protein diet and muscle mass (21).

In 1886, Max Jaffe (1841–1911) noticed that creatinine in contact with picric acid in an alkaline solution developed an orange-red color, proportional to creatinine concentration (22). Years later, in 1914, based on the Jaffe's experiment, Otto Folin measured the creatinine in deproteinized blood, incorporating Jaffe's reaction to the clinical diagnosis (23). By this time, creatinine had been identified as a substance removed only by filtration, the concept of clearance was introduced and widely applied in his studies by Homer Smith, and the determination of serum creatinine was available. All the conditions were met for using CrCl as a proxy for GFR in clinical practice.

At present, creatinine is one of the most frequent laboratory determinations: easy to perform, available almost everywhere, and cheap. It can be determined by enzymatic or colorimetric methods. However, as every analyte, serum creatinine measure is exposed to random error (performed by the operator) and systematic error (depending on the material, the instrument, and the process). Standardizing its determination as a method with traceable calibration to isotope dilution mass spectrometry (IDMS) reduces biases, improving the accuracy of creatinine determination (24, 25).

Isolated creatinine is not a good marker to evaluate kidney function, as it increases when GFR is around 50 ml/min or below. Creatinine clearance is better to estimate GFR, but it has some disadvantages when evaluating kidney function. Creatinine is not excreted only by glomerular filtration, as a small fraction is secreted at the tubular level. This fraction increases as kidney function decreases and cannot be calculated individually (21). Also, in advanced CKD, the intestinal microbiota contributes to degrading creatinine, and this proportion also cannot be estimated (26). Therefore, when CKD is present, CrCl tends to overestimate GFR, and the difference increases as kidney function decline. This fact moved to search mathematical formulas based on creatinine and demographic factors, such as age, sex, body mass index, and race, to estimate GFR (eGFR).

Despite the limitations described, creatinine continues to be the most frequent marker used in the clinic to assess the function of kidney.

In some situations, a 24-h urine collection is mandatory, and the measured creatinine clearance is preferred in some patient groups to avoid misinterpretations. This is the case of a very low protein intake, such as vegetarians, high protein intake, creatine supplementation, diet rich in meat, some muscle mass abnormalities (malnutrition, amputation, and loss of muscle mass), rapid change in kidney function, before starting dialysis or in children and pregnant women (27, 28).

The Development of Equations, Based on Creatinine, for Measuring eGFR

Equations to estimate GFR are widely used in day-to-day practice. More than 70 have been developed. In this publication, the most used and recent will be detailed.

One of the first ones was the Cockcroft–Gault (C–G) equation, available since the mid-1970s, that (or which) includes creatinine, sex, and weight, and is not adjusted for the body surface area (**Table 1**) (29). However, this equation correlates more to CrCl than to GFR. Besides that, the creatinine method used in the development of the C–G equation is no longer in use, and samples from the study are not available to compare the results to standardized creatinine values (30). Anyway, this equation has been and continues to be widely utilized, in part because many pharmacokinetic studies had been performed in the previously used C–G equation, before the standardization of serum creatinine traceable to IDMS (31, 32).

TABLE 1 | Most used creatinine and cystatin C equations to estimate glomerular filtration rate (eGFR).

Cockcroft-Gault equation
Creatinine Clearance = $\frac{140-\text{age (years)} \times \text{weight (kg)}}{72 \times \text{serum creatinine (mg/dl)}}$ x 0.85 (if female)
MDRD-4 (simplified)
Estimated Glomerular Filtration Rate (mL/min/1.73 m^2) = = 175 (Serum Creatinine in mg/dl × 0.011312)$^{-1.154}$ × (age in years)$^{-0.203}$ × (0.742 if female) × (1.212 if African American/black)
CKD-EPI (2009)
Estimated GFR = 141 x min(S_{Cr}/κ, 1)$^{\alpha}$ x max(S_{Cr} /κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if Black] SCr (standardized serum creatinine) = mg/dL., K = 0.7 (females) or 0.9 (males), α = −1.329 (female) or−0.411 (male), Min = indicates the minimum of S_{Cr}/K or 1, max = indicates the maximum of S_{Cr}/K or 1, Age = Years
FAS (2016)
1) Estimated GFR = 107.3/(SCr/ *Q*) for age ≤ 2 to ≤ 40 years 2) Estimated GFR = 107.3/(Scr/ *Q*) x 0.988 $^{(age-40)}$ for age > 40 years *Q*: the mean or median SCr value for age/sex-specific healthy populations
CKD-EPI cystatin C equation
Estimated Glomerular Filtration Rate (mL/min/1.73 m^2) = = 133 × min(Scys/0.8, 1)$^{-0.499}$ × max (Scys/0.8, 1)$^{-1.328}$ × 0.996^{Age} [× 0.932 if female] Scys = serum cystatin C, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scys/κ or 1
CKD-EPI creatinine-cystatin C
Estimated Glomerular Filtration Rate (mL/min/1.73 m^2) = 135 × min(Scr/κ, 1)$^{\alpha}$ × max(Scr/κ, 1)$^{-0.601}$ × min(Scys/0.8, 1)$^{-0.375}$ × max(Scys/0.8, 1)$^{-0.711}$ × 0.995^{Age} [× 0.969 if female] [× 1.08 if black] Scr = serum creatinine, Scys = serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Similar equations require other data like patient height and/or weight; many times this information is not recorded or correctly recorded, favoring erroneous results.

In 1999, Levey and associates developed seven equations applying a regression model to predict eGFR, using data of 1,628 patients enrolled in the baseline period of the Modification of Diet in Renal Disease (MDRD) study. The equation that gave the best agreement with iothalamate-measured GFR was the six variable equation, valid for a standard body surface of 1.73 m^2 (33):

GFR = 170 × [PCr]-0.999 × [Age]-0.176 × [0.762 if women] × [1.180 if African American/black] × [SUN]-0.170 × [Alb]+0.318

In 2000, Levey and coworkers proposed the simplified four-variable MDRD equation that correlates very well with the six-parameter equation proposed before (**Table 1**) (34). This

formula, originally defined for serum creatinine measured by the old Jaffe method, was re-expressed with the serum creatinine calibrated to an assay traceable to IDMS in 2006 (35). A recommendation to convert Jaffe-measured creatinine into reference method-based procedures (SCr_Jaffe × 0.95 @ SCr_enzyme) if the reference procedure used for the calibration was IDMS has been proposed but a full agreement for doing so has not arisen (25).

The MDRD GFR has little bias compared with measured GFR with urinary clearance of iothalamate under 60 ml/min/1.73 m^2, but underestimated the measured GFR at higher levels (36). These results are expected, as any equation reflects the characteristics of the population from which it derives; and the MDRD study was performed on the population with CKD.

In the year 2009, an improved creatinine-based equation, the CKD–EPI Collaboration (Chronic Kidney Disease Epidemiology Collaboration) was published, based on new methods for measuring creatinine, valid for all stages of renal insufficiency (**Table 1**). To work out the CKD–EPI equation, the authors took data from 8,254 participants of 10 studies (development set), 3,896 subjects from 16 studies (validation set) and, 16,032 individuals from NHANES (National Health and Nutritional Survey) for prevalence. The new equation performed significantly better than the MDRD study equation, especially at higher GFR, with lesser bias, improved precision, and greater accuracy (37).

This equation is still the most accurate GFR estimating equation evaluated in large diverse populations, applicable for the general clinical use. It provides lower estimates of the prevalence of decreased eGFR, and is useful as a trial measure for decreased eGFR and to replace the MDRD Study equation for routine reporting of serum creatinine-based eGFR by clinical laboratories (38).

A systematic search of MEDLINE, between 1999 and 21 October 2011, was performed by Earley et al. to review the GFR estimating equations performance; 12 studies were selected. In those from North America, Europe, and Australia, the authors corroborated, once again, that the CKD–EPI equation performed better at higher GFRs (> 60 ml/min/1.73 m^2) and the MDRD equation behaved better at lower GFRs (< 60 ml/min/1.73 m^2). In Asian or African populations, neither equation worked as well as in the other regions (39).

KDIGO CKD Guidelines recommend clinical laboratories to report eGFR in adults using the 2009 CKD–EPI equation (7), provided creatinine determination is traceable to IDMS.

The inclusion of race in the eGFR equation has been questioned, even though, in adults, age, sex, weight, height, and race are surrogates of muscle mass. In the original MDRD and CKD–EPI equations, the inclusion of race (Black/non-Black) improved accuracy (40). It has been argued that to exclude race from the eGFR equations would provoke a systematic under interpretation of measured GFR. To disclosure about the use of race when estimating GFR, or to accept denial to identify race from the patient, or to use a cystatin C confirmatory test has been proposed as a way to overcome the conflict (41, 42). In September 2021, the National Kidney Foundation and the American Society of Nephrology Joint Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Diseases recommended a new 2021 CKD-EPI creatinine eGFR equation which does not include race to estimate GFR (**Table 1**). They recommend for the United States immediate implementation of the CKD-EPI creatinine equation refit without the race variable in all laboratories, and to facilitate increased, routine and timely use of cystatin C, as combining creatinine and cystatin C is more accurate (43).

Finally, in 2016, Pottel et al. developed a novel equation, the full age spectrum (FAS) equation, to estimate the GFR across all over the age spectrum since available equations lack continuity with aging (the Schwartz equation for pediatrics, the CKD–EPI equation for adults under 70 years age, and the BIS-1 for older than 70 years old). This new equation is normalized on serum Cr (SCr/Q) for age (children and adolescents) and gender (adolescents and adults), being Q the median serum Cr from a specific healthy subpopulation. In the validation study, 6,870 healthy and kidney disease caucasian and from the non-African origin individuals, of whom 765 were children and adolescents <18 years old and 1,748 elderly higher than 70 years old, participated. For validation, measured GFR was performed using inulin or iothalamate or iohexol clearance (**Table 1**) (44). The FAS equation that can be used in ages <2–100 years old, resulted less biased and more accurate than the Schwartz equation for children and adolescents, and less biased and as accurate as the CKD–EPI equation for adults under or over 70 years old.

The Development of Equations Using Cystatin C

Cystatin C was described for the first time in 1961 (45). As creatinine, it is an endogenous marker. It is a low molecular weight protein (13 kD) and consists of a chain of 120 amino acids. Produced constantly by all nucleated cells of the body, it filters freely through the glomerulus and is totally reabsorbed and catabolized by the proximal tubular cells. Muscle mass, age, sex, or diet do not affect its concentration; these characteristics make Cystatin C useful in groups with reduced muscle mass (46, 47).

To improve the accuracy of eGFR several equations have now been developed using either cystatin C alone or cystatin C in combination with creatinine. Cystatin C-based equations have advantages over the creatinine-based equation as they are less influenced by age, sex, and race (48). The 2012 CKD–EPI creatinine-cystatin C equation is more accurate than the 2009 CKD–EPI creatinine and 2012 CKD–EPI cystatin C equations and it is useful as a confirmatory test for decreased eGFR as determined by serum creatinine-based eGFR (**Table 1**) (49).

Despite its greater usefulness, cystatin C has not displaced creatinine for GFR estimation in clinical practice, possibly due to its higher cost and lower availability.

Clinical Situations Where mGFR Is Needed

In some clinical situations, such as patients with anorexia nervosa, cirrhosis, obesity, evaluation of living kidney donors, prescribing nephrotoxic drugs with a narrow therapeutic window, pharmacokinetic studies of drugs excreted by the kidney, or any situation in which eGFR is unreliable, it is reasonable to measure GFR (mGFR), despite the added cost

and time and resources consuming (7, 50–52). At present, iothalamate or 51Cr-EDTA or 99Tcm-DTPA urinary clearance or 99Tcm-DTPA or iohexol plasma clearance are the accurate methods for determining mGFR (52–55). In patients with large edema or ascites, urinary clearance should be employed (55).

Iohexol, a low-cost non-toxic non-ionic contrast agent, has some advantages for the plasma clearance such as simplicity, low cost, stability, and low interlaboratory variation. Besides that, it is not radioactive, is excreted almost exclusively by the kidney, is neither secreted nor reabsorbed at the tubular level, has low protein binding, and correlates with inulin renal clearance (56, 57). It is contraindicated in patients with allergy to iodine.

CONCLUSION

From the knowledge that emerges from renal physiology, laboratory medicine, epidemiology, and biostatistics, have emerged equations that constitute tools not only for the clinical care of patients, but also to establish the prevalence of CKD, and consequently implementing the public health policies aimed to reduce it.

The inclusion of the automatic calculation of GFR in the laboratory reports of creatinine determinations constitutes a useful tool for daily practice, and from public health perspectives for population screening for CKD.

CKD–EPI Study equation continues to be the most used in general practice and from public health perspective. Recently, the FAS equation emerged as a promising option to estimate eGFR for all ages (from 2 to 100 years old).

Further improvement in GFR estimating equations will require development in more broadly representative populations, such as diverse racial and ethnic groups, use of multiple filtration markers, and evaluation using statistical techniques to compare eGFR to mGFR.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. American Society of Nephrology (ASN), European Renal Association – European Dialysis and Transplant Association (ERA-EDTA), International Society of Nephrology (ISN). *The Hidden Epidemic: Worldwide, Over 850 Million People Suffer From Kidney Diseases.* (2018). Available online at: https://www.asn-online.org/news/2018/0626-Joint_Hidden_Epidem.pdf (accessed July 22, 2021).
2. *The World bank Total Population*. Available online at: https://data.worldbank.org/indicator/SP.POP.TOTL (accessed July 22, 2021).
3. *Who Fact sheets Diabetes*. Available online at: http://www.who.int/news-room/fact-sheets/detail/diabetes (accessed July 22, 2021).
4. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* (2016) 388:1459–544. doi: 10.1016/S0140-6736(16)31012-1
5. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet.* (2018) 392:2052–90. doi: 10.1016/S0140-6736(18)31694-5
6. Matsushita K, Coresh J, Sang Y, Chalmers J, Fox C, Guallar E, et al. Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes; a collaborative meta-analysis of indivividual participant data. *Lancet Diabetes Endocrinol.* (2015) 3:514–25. doi: 10.1016/S2213-8587(15)00040-6
7. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl.* (2013) 3:1–150. doi: 10.1038/kisup.2012.73
8. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* (2002) 39(2 Suppl. 1): S1–266.
9. Wang Y, Chen X, Caballero B, Cheskin LJ. Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int.* (2008)73:19–33. doi: 10.1038/sj.ki.5002586
10. Vassalotti JS, Stevens LA, Levey AS. Testing for chronic kidney disease: a position statement from the National Kidney Foundation. *Am J Kidney Dis.* (2007) 50:169–80. doi: 10.1053/j.ajkd.2007.06.013
11. Levin A, Stevens PE. Early detection of CKD: the benefits, limitations and effects on prognosis. *Nat Rev Nephrol.* (2011) 7:446–57. doi: 10.1038/nrneph.2011.86
12. Schlipak MG, Tummalapalli SL, Boulware LE, Winkelmayer WC, Zoungas S. for Conference Participants. The case for early identification and intervention of chronic kidney disease: conclusions from a Kidney Disease: Improving Global Outomes (KDIGO) Controversies Conference. *Kidney Int.* (2021) 99:34–47. doi: 10.1016/j.kint.2020.10.012
13. Ludwig C. *De viribus physicis secretionen urinae adjuvantibus*. Marburg: Elwert (1842).
14. Davies J, Gottschalk CW, Haberle DA, Thurau K. Carl Ludwig's revolutionary concept of renal function. *Kidney Int.* (1994) 46:1–23.
15. Jamison RL. Resolving an 80-yr. -old controversy: the beginning of the modern era of renal physiology. *Adv Physiol Educ.* (2014) 38:286–95. doi: 10.1152/advan.00105.2014
16. Cushny AR. The secretion of the urine. *Nature.* (1917) 99:304.
17. Wearn JT, Richards AN. Observations of the composition of glomerular urine, with particular reference to the problem of reabsorption in the renal tubules. *Am J Physiol.* (1924) 71:0209–227. doi: 10.1152/ajplegacy.1924.71.1.209
18. Rehberg PB. Studies on kidney function. I The rate of filtration and reabsorption in the human kidney. *Biochem J.* (1926) 20:447–60. doi: 10.1042/bj0200447
19. Moller E, McIntoshJF, Van Slike DD. Studies on urea excretion. II. Relationship between urine volume and the ratio of urea excretion by normal adults. *J Clin Invest.* (1928) 6:427–65. doi: 10.1172/JCI100206
20. Smith HW. Note on the interpretation of clearance methods in the diseased kidney. *J Clin Invest.* (1941) 20:631–5. doi: 10.1172/JCI101256
21. Perrone RD, Madias NE, Levey AS. Serum Creatinine as an Index of Renal Function: New Insights into Old Concepts. *Clin Chem.* (1992) 38:1933–53. doi: 10.1093/clinchem/38.10.1933
22. Jaffe M. Über den Niederschlag, welchen Pikrinsäre in normalem Harn erzeugt und über eine neue Reaction des Kreatinins. *Zeitschrift für physiologische Chemie.* (1886) 10:391–400. doi: 10.1515/bchm1.1886.10.5.391
23. Shaffer PA. *Otto Folin 1867-1934*. Washington, DC: National Academy of Sciences (1952). p. 47–82.
24. Cook JG. Factors influencing the assay of creatinine. *Ann Clin Biochem.* (1975) 12:219–32. doi: 10.1177/000456327501200162
25. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creatinine measurement: a report from the laboratory working group of the National kidney disease Education

program. *Clin Chem.* (2006) 52:5–18. doi: 10.1373/clinchem.2005.0525144
26. Papadakis MA, Arieff AI. Unpredictability of clinical evaluation of renal function in cirrhosis. Prospective study. *Am J Med.* (1987) 82:945–52. doi: 10.1016/0002-9343(87)90156-2
27. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function: measured and estimated glomerular filtration rate. *N Engl J Med.* (2006) 354:2473–83. doi: 10.1056/NEJMra054415
28. Lopes van Balen VA, van Gansewinkel TAG, de Haas S, Spaan JJ, Ghossein-Doha C, van Kuijk SMJ, et al. Maternal kidney function during pregnancy: systematic review and meta-analysis. *Ultrasound Obstet Gynecol.* (2019) 54:297–307. doi: 10.1002/uog.20137
29. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* (1976) 16:31–41. doi: 10.1159/000180580
30. Jones G. Estimating renal function for drug dosing decisions. *Clin Biochem Rev.* (2011) 32:81–8.
31. National Kidney Disease Education Program: NKDEP CKD and Drug Dosing: Information for Providers. Available online at: http://www.nkdep.nih.gov/professionals/drug-dosing-information.htm#cockcroft-gault (accessed August 31, 2021).
32. Matzke GR, Aronoff GR, Atkinson AJ, Bennett WM, Decker BS, Eckardt KW, et al. Drug dosing consideration in patients with acute and chronic kidney disease-a clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int.* (2011) 80:1122–37. doi: 10.1038/ki.2011.322
33. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, et al. more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
34. Levey AS, Green T, Kusek J, Beck GA. simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol.* (2000) 11:155A.
35. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med.* (2006) 145:247–54. doi: 10.7326/0003-4819-145-4-200608150-00004
36. Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RJ, et al. Evaluation of the modification of diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol.* (2007) 18:2749–57. doi: 10.1681/ASN.2007020199
37. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, et al. New equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
38. Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. *Am J Kidney Dis.* (2010) 55:622–7. doi: 10.1053/j.ajkd.2010.02.337
39. Earley A, Miskulin D, Lamb EJ. S Levey AS, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. *Ann Intern Med.* (2012) 156:785–95. doi: 10.7326/0003-4819-156-11-201203200-00391
40. Levey AS, Titan SM, Powe NR, Coresh J, Inker LA. Kidney disease, race, and GFR estimation. *Clin J Am Soc Nephrol.* (2020) 15:1203–12. doi: 10.2215/CJN.12791019
41. Duggal V. Thomas I-Chun, Montez-Rath ME, Chertow GM, Tamura MK. National Estimates of CKD prevalence and potential impact of estimating glomerular filtration rate without race. *J Am Soc Nephrol.* (2021) 32:1454–63. doi: 10.1681/ASN.2020121780
42. Hsu C, Yang W, Parikh RV, Anderson AH, Chen TK, Cohen DL, et al. Race, genetic ancestry, and estimating kidney function in CKD. *N Engl J Med.* (2021) 385:1750–60. doi: 10.1056/NEJMoa2103753
43. Delgado C, Baweja M, Crews DC, Eneanya ND, Gadegbeku CA, Inker LA, et al. A Unifying Approach for GFR Estimation: Recommendations of the NKF-ASN Task Force on Reassessing the Inclusion of Race in Diagnosing Kidney Disease. *Am J Kid Dis.* (2021) 22:S0272-6386(21)00828-3. doi: 10.1053/j.ajkd.2021.08.003
44. Pottel H, Hoste L, Dubourg L, Ebert N, Schaeffner E, Eriksen BO, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant.* (2016) 31:798–806. doi: 10.1093/ndt/gfv454
45. Butler EA, Flynn FV. The occurrence of part-gamma protein in urine: a new protein abnormality. *J Clin Path.* (1961) 14:172–8. doi: 10.1136/jcp.14.2.172
46. Abrahamson M, Olafsson I, Palsdottir A, Ulvsbäck M, Lundwall A, Jensson O, et al. Structure and expression of the human cystatin C gene. *Biochem J.* (1990) 268:287–94. doi: 10.1042/bj2680287
47. Filler G, Bokenkamp A, Hofmann W, Bricon TL, Martinez-Bru C, Grubb A. Cystatin C as a marker of GFR – history, indications, and future research. *Clin Biochem.* (2005) 38:1–8. doi: 10.1016/j.clinbiochem.2004.09.025
48. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis.* (2008) 51:395–406. doi: 10.1053/j.ajkd.2007.11.018
49. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* (2012) 367:20–9. doi: 10.1056/NEJMoa1114248
50. Ebert E, Bevc S, Bökenkamp A, Gaillard F, Hornum M, Jager KJ, et al. Assessment of kidney function: clinical indications for measured GFR. *Clin Kidney J.* (2021) 14:1861–70. doi: 10.1093/ckj/sfab042
51. European Medicines Agency. *Guideline on the Evaluation of the Pharmacokinetics of Medicinal Products in Patients With Decreased Renal Function.* London: European Medicines Agency (2014). Available online at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/02/WC500162133.pdf (accessed November 11, 2021).
52. Soveri I, Berg UB, Björk J, Elinder CG, Grubb A, Mejare I, et al. Measuring GFR: a systematic review. *Am J Kidney Dis.* (2014) 64:411–24. doi: 10.1053/j.ajkd.2014.04.010
53. Agarwal R, Delanaye P. Glomerular filtration rate: when to measure and in which patients? *Nephrol Dial Transpl.* (2019) 34:2001–7. doi: 10.1093/ndt/gfy363
54. Vidal-Petiot E, Courbebaisse M, Livrozet M, Corrégé G, Rusu T, Montravers F, et al. Comparison of ^{51}Cr-EDTA and ^{99m}Tc-DTPA for glomerular filtration rate measurement. *J Nephrol.* (2021) 34:729–37. doi: 10.1007/s40620-020-00932-9
55. Delanaye P, Ebert N, Melsom T, Gaspari F, Mariat C, Cavalier E, et al. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: How to measure glomerular filtration rate with iohexol? *Clin Kidney J.* (2016) 9:682–99. doi: 10.1093/ckj/sfw070
56. Gaspari F, Perico N, Ruggenenti P, Mosconi L, Amuchastegui CS, Guerini E, et al. Plasma clearance of nonradioactive Iohexol as a measure of glomerular filtration pate1. *J Am Soc Nephrol.* (1995) 6:257–63. doi: 10.1681/ASN.V62257
57. Brown SC, O'Reilly PH. Iohexol clearance for the determination of glomerular filtration rate in clinical practice: evidence for a new gold standard. *J Urol.* (1991) 146: 675–9. doi: 10.1016/S0022-5347(17)37891-6

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 29 November 2021
doi: 10.3389/fmed.2021.760391

Trajectory of Estimated Glomerular Filtration Rate and Malnourishment Predict Mortality and Kidney Failure in Older Adults With Chronic Kidney Disease

Shuo-Chun Weng[1,2,3], *Chyong-Mei Chen*[4], *Yu-Chi Chen*[5], *Ming-Ju Wu*[1,2,6,7,8] *and Der-Cherng Tarng*[3,9,10,11]*

[1] College of Medicine, National Chung Hsing University, Taichung, Taiwan, [2] Center for Geriatrics and Gerontology, Division of Nephrology, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, [3] Institute of Clinical Medicine, School of Medicine, College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [4] Institute of Public Health, College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [5] Institute of Clinical Nursing, College of Nursing, National Yang Ming Chiao Tung University, Taipei, Taiwan, [6] School of Medicine, Chung Shan Medical University, Taichung, Taiwan, [7] Rong Hsing Research Center for Translational Medicine, Institute of Biomedical Science, College of Life Science, National Chung Hsing University, Taichung, Taiwan, [8] Graduate Institute of Clinical Medical Science, School of Medicine, China Medical University, Taichung, Taiwan, [9] Department and Institute of Physiology, National Yang Ming Chiao Tung University, Taipei, Taiwan, [10] Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [11] Center for Intelligent Drug Systems and Smart Bio-devices (IDS2B), National Yang Ming Chiao Tung University, Hsinchu, Taiwan

OPEN ACCESS

Edited by:
Ana Cusumano, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Argentina

Reviewed by:
María Azancot, Vall d'Hebron University Hospital, Spain
Jonathan P. Troost, University of Michigan, United States

***Correspondence:**
Der-Cherng Tarng
dctarng@vghtpe.gov.tw

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *18 August 2021*
Accepted: *08 November 2021*
Published: *29 November 2021*

Citation:
Weng S-C, Chen C-M, Chen Y-C, Wu M-J and Tarng D-C (2021) Trajectory of Estimated Glomerular Filtration Rate and Malnourishment Predict Mortality and Kidney Failure in Older Adults With Chronic Kidney Disease. Front. Med. 8:760391. doi: 10.3389/fmed.2021.760391

Objective: The trajectory patterns of estimated glomerular filtration rates (eGFR) in chronic kidney disease (CKD) older adults with malnourishment and their association with subsequent patient outcomes have not been elucidated. We aimed to assess the eGFR trajectory patterns for predicting patient survival and kidney failure in the elderly without or with malnourishment.

Materials and Methods: Based on a prospective longitudinal cohort, CKD patients aged 65 years or older were enrolled from 2001 to 2013. Among the 3,948 patients whose eGFR trajectory patterns were analyzed, 1,872 patients were stratified by the absence or presence of malnourishment, and 765 patients were identified and categorized as having malnourishment. Four eGFR trajectory patterns [gradual decline (T0), early non-decline and then persistent decline (T1), persistent increase (T2), and low baseline and then progressive increase (T3)] were classified by utilizing a linear mixed-effect model with a quadratic term in time. The malnourishment was defined as body mass index <22 kg/m^2, serum albumin <3.0 mg/dL, or Geriatric Nutritional Risk Index (GNRI) <98. This study assessed the effectiveness of eGFR trajectory patterns in a median follow-up of 2.27 years for predicting all-cause mortality and kidney failure.

Results: The mean age was 76.9 ± 6.7 years, and a total of 82 (10.7%) patients with malnourishment and 57 (5.1%) patients without malnourishment died at the end of the study. Compared with the reference trajectory T0, the overall mortality of T1 was markedly reduced [adjusted hazard ratio (aHR) = 0.52, 95% confidence interval (CI) 0.32–0.83].

In patients with trajectory, T3 was associated with a high risk for kidney failure (aHR = 5.68, 95% CI 3.12–10.4) compared with the reference, especially higher risk in the presence of malnourishment. Patients with high GNRI values were significantly associated with a lower risk of death and kidney failure, but patients with malnourishment and concomitant alcohol consumption had a higher risk of kidney failure.

Conclusions: Low baseline eGFR and progressively increasing eGFR trajectory were high risks for kidney failure in CKD patients. These findings may be attributed to multimorbidity, malnourishment, and decompensation of renal function.

Keywords: eGFR trajectory, kidney failure, malnourishment, mortality, older adults

INTRODUCTION

Longitudinal eGFR change in patients with CKD is often nonlinear, and most people with CKD have major clinical events and die before reaching kidney failure. In previous observational studies, patients with CKD experienced the possibility of non-progression of renal function, stable kidney disease with accelerated deterioration, and relentlessly fast eGFR decline into kidney failure (1–3). However, a paradoxical association exists between high eGFR and fast eGFR decline, which were found to be correlated with increased risk of death, but were associated with a comparatively lower risk of developing kidney disease (2, 4). For the above reasons, other parameters, such as serum creatinine, eGFR value, nutritional assessment, and severity of comorbid conditions are repeatedly measured to predict patients' outcomes. Traditional methods, such as baseline eGFR by category, absolute change in eGFR value, annual eGFR decline or annual percentage change of eGFR, the velocity of eGFR slopes, and eGFR variability, have been widely used for predicting cognitive deterioration, cardiovascular risk, renal outcome, and patient mortality (5–11). However, a more comprehensive picture of disease progression can be obtained using the eGFR trajectory pattern classification, which has been proven superior to baseline eGFR since it can capture the whole process of eGFR measurements and can take non-linear patterns into account (12), whereas baseline eGFR at cohort entry or longitudinal eGFR values showing linear trends provide more limited information.

Malnourishment, which was caused by CKD, respiratory disease, cancer, and cardiovascular disease, was reported to possibly contribute to high eGFR mortality, especially in older adults (4, 13). The main reasons for developing malnourishment were comorbid illnesses, oxidative and carbonyl stress, nutrient loss through diseases, anorexia and low nutrient intake, uremic toxins, and a decreased clearance of inflammatory cytokines (14). More importantly, older adults have age-related changes in appetite, swallowing, and dental problems, and those issues are predisposed to decreased food intake and malnourishment, especially inadequate fluid or protein intake. Thus, age-related multimorbidity and loss of muscle mass would tend to contribute to a progressively increasing eGFR trajectory and make an assessment of long-term clinical outcomes based on short-term measurements of renal function more difficult (15, 16). Besides, the U-shaped relationship between low body mass index (BMI) and sarcopenic obesity has been strongly associated with all-cause and cardiovascular mortality in older adults (17, 18), and our previous study revealed that the increased risk of mortality in subjects with high eGFR was primarily attributable to the presence of malnutrition-inflammation-cachexia syndrome (4). However, few studies have discussed the relationships among eGFR trajectories, BMI, and nutritional status and their effects on clinical outcomes.

Clinically, great diversity in longitudinal eGFR measurements exists. These patterns reveal different clinical pictures of kidney function. Taking these characteristics of longitudinal eGFR profiles into account would provide more information on the condition of kidneys compared with using baseline eGFR or decline rate. Therefore, we conducted a prospective longitudinal cohort study using the eGFR trajectory to predict patient survival and renal outcome in CKD older adults without or with malnourishment.

METHODS

Patients and Data Sources

Data from the CKD cohort were obtained from patients' medical records at Taichung Veterans General Hospital (TCVGH_ CKD cohort, **Figure 1**). All registration data were obtained from the Chronic Kidney Disease division of the Bureau of Health Promotion, Ministry of Health and Welfare (CKDBHPDH) (19). CKD was defined as eGFR $<$ 60 ml/min more than 3 months, urine albumin/creatinine ratio $>$30 mg/g (20), urine protein/creatinine ratio $>$0.2 mg/g (21) or abnormal kidney image. In the TCVGH_CKD cohort, we identified men and women older than 65 years of age with CKD (n = 3,948) from five counties and cities in central Taiwan from December 1, 2001, to July 31, 2013. Among the 3,948 patients, 2,076 patients without complete data of malnourishment criteria were excluded. 1,872 participants whose eGFR trajectory patterns were analyzed and were stratified by the absence or presence of malnourishment, and 765 patients were identified and categorized as having malnourishment. The patients in the TCVGH_CKD cohort had at least 3 outpatient eGFR records per year, based on data obtained from the Taiwan Society of Nephrology (TSN). However, before January 01, 2014, serum creatinine was measured by the Jaffe method using a Beckman Synchron CX5 analyzer (USA) calibrated under the standards of

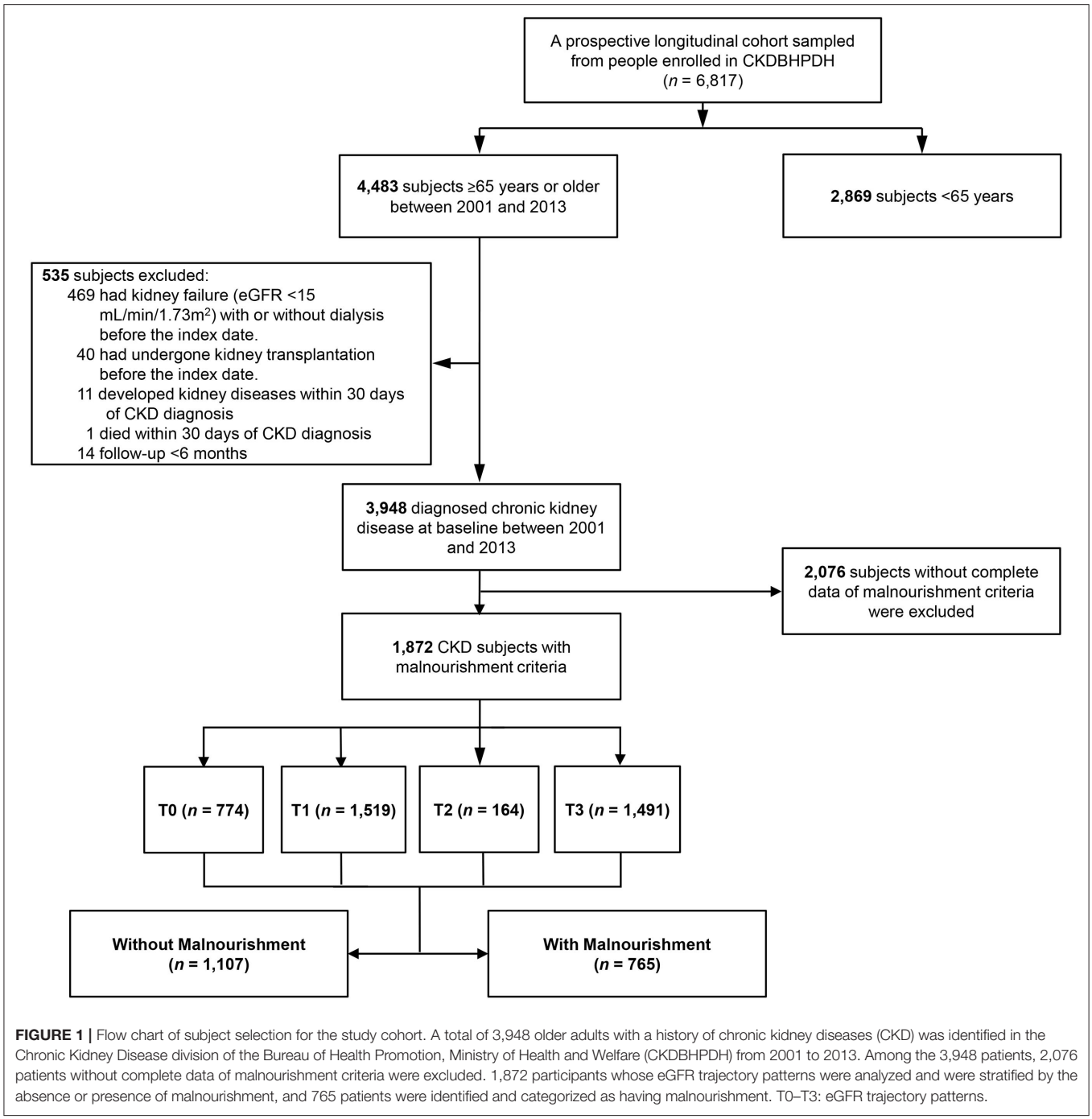


FIGURE 1 | Flow chart of subject selection for the study cohort. A total of 3,948 older adults with a history of chronic kidney diseases (CKD) was identified in the Chronic Kidney Disease division of the Bureau of Health Promotion, Ministry of Health and Welfare (CKDBHPDH) from 2001 to 2013. Among the 3,948 patients, 2,076 patients without complete data of malnourishment criteria were excluded. 1,872 participants whose eGFR trajectory patterns were analyzed and were stratified by the absence or presence of malnourishment, and 765 patients were identified and categorized as having malnourishment. T0–T3: eGFR trajectory patterns.

the Chinese National Laboratory Accreditation program. After January 01, 2014, the enzymatic method (CYGNUS AUTO CRE) and spectrophotometry were used. Patients were excluded if they had undergone kidney transplantation or had irreversible kidney failure with or without dialysis before the index date. The TCVGH_CKD database included patients with early CKD (CKD stage 1, 2, 3A) and pre-kidney failure (CKD stage 3B, 4, 5), and all participants were followed up for longer than 6 months, until February 07, 2014, to prevent lead-time bias.

Patient and Public Involvement Statement

Older adults with CKD are often undernourished and physically inactive, which contributes to sarcopenia and frailty. The trajectory of eGFR is a potential surrogate marker to demonstrate the causal relationship among muscle mass, aging changes in the kidneys, renal survival, and patient mortality. Outcome measurements and study design were approved by the institutional review board of TCVGH informed by patients' priorities, experience, and preferences according to the

Declaration of Helsinki. Using administration data from the CKDBHPDH, a prospective cohort was selected and enrolled. All subjects gave their informed consent for inclusion before they participated in the study. Although informed consent was required, multidisciplinary and geriatric care did not interfere with clinical decisions related to patient care. Whether written informed consent was given by participants for their clinical records to be used in this study, every consent was obtained before the patient records/information was anonymized and de-identified before analysis. The results will be disseminated to study participants if the dissertation would be accepted. The study protocol was affirmed by the Ethics Committee of Taichung Veterans General Hospital and Taipei City Hospital.

Definition of eGFR Trajectory Patterns

Each patient was classified into one of four groups based on the four-variable Modification of Diet in Renal Disease (MDRD) Study equation (22, 23). Those eGFR data were obtained prospectively, and the number of eGFR measurements is various with ≥3 before enrollment. To stratify the eGFR trajectory patterns, we first applied a linear mixed-effects model with a quadratic term in time for repeatedly measured eGFR values (polygonal lines in **Supplementary Figure 1**) (24). More specifically, the eGFR of each patient is modeled as a quadratic function of time to examination since treatment with all terms having random effects. In addition to the estimation of the fixed effects, we also obtained the empirical Bayes estimates of each patient's random effects, which are distributed random variables with a mean zero. By plugging these estimates, a patient's trajectory is fitted by the polynomial of degree 2 with the corresponding estimated random effects and fixed effects as coefficients. Based on that, we can classify an individual into one of the 4 eGFR trajectories. eGFR trajectory pattern 1 (T0), which is characterized by the baseline eGFR 27.9 ± 9.8 and a median 9.25-year later the eGFR estimated as 11.3 ± 10.3 ml/min/1.73m^2 per year (25), was defined to be stably slow progression accounting for 19.6% of patients (**Figure 2**). Thirty-five empirical trajectories of eGFR_MDRD randomly selected from each group are drawn in the following figure (polygonal lines in **Supplementary Figure 1**) combining with the classified eGFR_MDRD trajectories for these 4 groups. Each smooth curve is the average of the observed trajectories in the group corresponding to the same color. It can be seen that the estimated and smooth trajectories fitted all patients' empirical eGFR trajectories well. Trajectory pattern 2 (T1), which is characterized by early non-decline (<-0.43 to −0.48 ml/min/1.73 m^2 per year) (2) and then persistent decline of more than −3 ml/min/1.73 m^2 per year, accounted for 38.4% of patients. The turning point of the eGFR trajectory was determined by the joint model using latent random effects (26). Trajectory pattern 3 (T2), which is characterized by persistently increasing eGFR without any decreases, accounted for 4.2% of patients. Trajectory pattern 4 (T3), which is characterized by an initial low eGFR of 20 ml/min/1.73 m^2 and mild progression (≥-2 ml/min/1.73 m^2 per year for every month of the period or ≥-1 ml/min/1.73 m^2 per year, which corresponded roughly to the average age-related decline in GFR) (1) in the first 5 years, followed by progressive increasing eGFR (T3) values without any decreases, accounted for 37.8% of patients. Sensitive analyses with the CKD-EPI equations (27) were also shown in **Figure 2**.

Assessment of Malnourishment

The clinical presence of malnourishment in older adults was determined based on the following indicators: body mass index <22 kg/m^2, serum albumin <3.0 g/dL, or GNRI < 98 (4). The GNRI formula is as follows: GNRI = (1.489 × albumin in gram per liter) + (41.7 × present/ideal body weight). The ideal body weight was calculated according to the Lorentz formula, which takes into account a patient's height and sex: for men, height – 100 – ([height – 150]/4); for women, height – 100 – ([height – 150]/2.5) (4, 28–30).

Availability of Data and Imputation

There are missing values for various variables in the judgment of malnourishment in 3,948 patients, and we listed the detailed numbers of patients with abnormal levels of albumin, BMI, or GNRI in **Supplementary Table 1**. All missing values were imputed using multiple imputations with the FCS (Fully Conditional Specification) imputation method (31). Ten imputations were performed. All the estimates and the corresponding standard error estimates in each imputation were properly combined.

Covariates

Covariates included age, gender, smoking, alcohol, initial eGFR, diabetes mellitus (DM), hypertension (HTN), cardiovascular diseases, malignancy, malnourishment components (4, 28), the classified eGFR trajectory patterns, serum uric acid, HbA1c, and medication. Baseline comorbidities were ascertained at the time of study entry. Comorbid conditions were self-reported by the patients or retrieved from electronic records. The presence of cardiovascular diseases, i.e., coronary artery disease, stroke, congestive heart failure, arrhythmia, and peripheral arterial disease, was determined using patients' medical records.

Study Outcomes

Mortality was obtained from the death registry system and coded from death certificates according to the International Classification of Diseases (ICD)-9 or ICD-10. The primary outcome was all-cause mortality, and the secondary outcome was kidney failure. Patients were identified as having kidney failure if they had undergone maintenance dialysis or short-term dialysis before death.

Statistical Analyses

Based on the empirical Bayes estimates of all random effects, each patient's eGFR profile was estimated. The results revealed four patterns of eGFR trajectories. Each patient was classified into one of the four trajectory patterns according to his/her estimated eGFR profile. To construct eGFR trajectories, the longitudinal eGFR pattern was followed for nearly 140 months (**Figure 2**). The baseline demographics are presented as number and percentage for categorical variables and as mean with standard deviation (SD) or median with interquartile range (IQR) for continuous variables. We compared the characteristics of patients

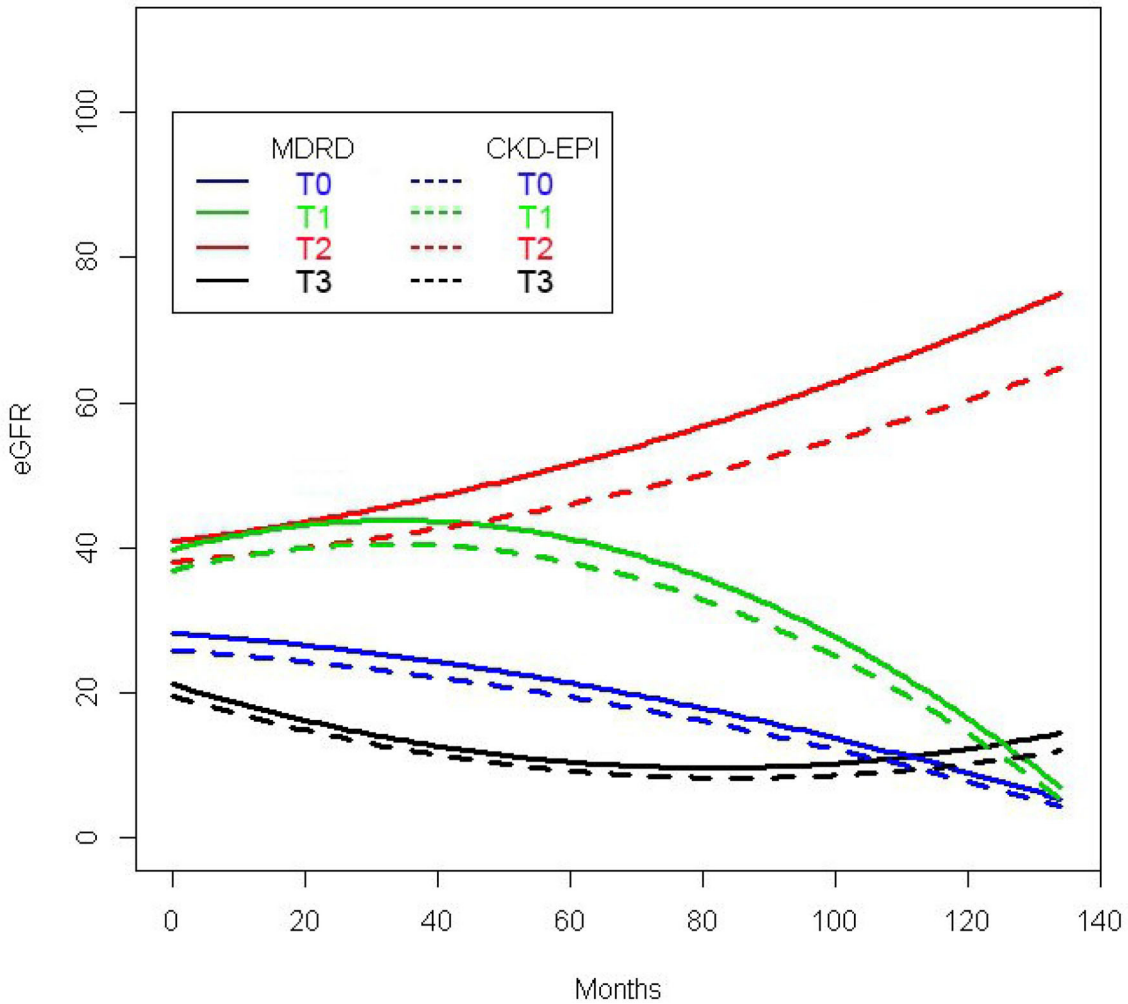


FIGURE 2 | Four eGFR trajectories in the CKD cohort. Pattern 1 (T0): solid blue line, gradual eGFR decline; pattern 2 (T1): solid green line, early non-decline and then persistent decline; pattern 3 (T2): solid red line, persistently increasing eGFR; pattern 4 (T3): solid black line, low baseline eGFR with the early decline and then progressively increasing eGFR. Four eGFR trajectories with the CKD-EPI equations were also shown (dotted blue, green, red, and black line).

with and without malnourishment and among the different eGFR trajectory patterns using a Chi-square test for categorical variables and one-way ANOVA for continuous variables. The *post-hoc* analysis was conducted to compare all pairs of subgroups. These calculations were conducted using SAS PROC MI and PROC MIANALYZE. Multinomial logistic regression analysis was applied to evaluate the associations between eGFR trajectory patterns and factors, including demographic variables, comorbid conditions, lifestyle, and nutritional status of older people. The primary outcome, all-cause mortality, and the secondary outcome, kidney failure, were analyzed using cause-specific hazard models for competing risk data. All variables, including the classification of eGFR trajectory patterns, were adjusted in the survival analysis. In all tests, a $p < 0.05$ was considered statistically significant. Statistical analyses were implemented using R statistical software, version 3.4.2, and SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Baseline Demographics

Based on a prospective longitudinal cohort (**Figure 1**), **Figure 2** shows 4 major eGFR profiles which were determined by calculating the average empirical Bayes estimates of all random effects in each patient. We further analyzed the demographic and clinical characteristics of the cohort according to each of the eGFR trajectory patterns (**Table 1**). Patients with persistent increasing eGFR (T2) were older than patients in the other subgroups, and the T1 group had a higher percentage of smoking and alcohol consumption after *post-hoc* test comparing all pairs of subgroups. Baseline eGFR in the T3 group was lower than that in the other subgroups, and a higher proportion of patients in the late stages of CKD were also found in the T3 group (**Table 1**).

Prevalence of nutrition-related risks, including low serum albumin, abnormal BMI, and low GNRI value in both the T2

TABLE 1 | Demographic and clinical characteristics of four eGFR patterns.

Parameter*	T0	T1	T2	T3	*p* value
Number of patients	***n* = 774**	***n* = 1,519**	***n* = 164**	***n* = 1,491**	
Demographic characteristics					
Age (years)	76.1 ± 7.0	76.3 ± 7.1	79.9 ± 6.9	75.0 ± 6.9	<0.001
Gender, male (%)	510 (65.9)	1,155 (76.0)	121 (73.8)	854 (57.3)	<0.001
Smoking (%)	289 (39.6)	622 (42.5)	57 (36.3)	494 (35.0)	<0.001
Alcohol (%)	186 (25.5)	446 (30.5)	41 (26.1)	326 (23.1)	<0.001
Diabetes mellitus (%)	311 (40.2)	552 (36.3)	44 (26.8)	649 (46.0)	<0.001
Hypertension (%)	538 (69.5)	1,097 (72.2)	108 (65.9)	1,110 (78.7)	<0.001
Cardiovascular diseases† (%)	103 (13.3)	201 (13.2)	28 (17.1)	159 (11.3)	0.108
Malignancy (%)	80 (11.0)	116 (7.9)	3 (7.3)	123 (8.7)	0.124
Baseline eGFR, ml/min per 1.73 m^2 by MDRD equation	27.9 ± 9.8	38.5 ± 13.2	58.0 ± 22.3	21.5 ± 14.4	<0.001
1. ≥90	0 (0.0)	5 (0.3)	10 (6.1)	3 (0.2)	<0.001
2. 60 to <90	7 (0.9)	84 (5.5)	63 (38.4)	12 (0.8)	
3. 30 to <60	289 (37.3)	1,092 (71.9)	78 (47.6)	372 (24.9)	
4. 15 to <30	420 (54.3)	291 (19.2)	11 (6.7)	439 (29.4)	
5. <15	58 (7.5)	47 (3.1)	2 (1.2)	665 (44.6)	
Malnourishment indicators					
Serum albumin <3.5 g/dL (%)	108 (13.9)	161 (10.6)	36 (21.7)	313 (21.0)	<0.001
BMI, kg/m^2	24.6 ± 3.7	24.7 ± 3.5	24.3 ± 4.4	24.5 ± 3.6	0.474
GNRI	105.9 ± 11.1	107.2 ± 10.6	101.4 ± 11.8	103.0 ± 11.4	<0.001
Other laboratory data					
Serum uric acid ≥7.2 mg/dL (%)	523 (67.6)	896 (59.0)	97 (59.3)	941 (63.1)	0.013
HbA1c ≥ 6.5% (%)	131 (16.9)	260 (17.1)	7 (4.3)	253 (17.0)	<0.001
Serum albumin, g/dL	3.9 ± 0.5	4.1 ± 0.5	3.8 ± 0.5	3.8 ± 0.5	<0.001
Medications, n (%)					
ACEIs/ARBs	497 (64.2)	992 (65.3)	116 (70.6)	854 (57.3)	0.001
Insulin	265 (34.2)	380 (25.0)	72 (43.8)	587 (39.4)	<0.001
OAD	271 (35.0)	501 (33.0)	24 (14.6)	558 (37.4)	<0.001
Antilipemic agents	172 (22.2)	374 (24.6)	72 (43.9)	362 (24.3)	0.016
Drugs for hypertension	559 (72.2)	1,104 (72.7)	136 (82.9)	1,145 (76.8)	0.024
Follow-up time, years, median (25th–75th percentile)	1.8 (1.1–4.5)	2.7 (1.4–4.8)	2.0 (1.3–3.6)	2.1 (1.1–4.0)	<0.001
Death (%)	102 (13.2)	95 (6.3)	7 (4.3)	129 (8.7)	<0.001

**Data are presented as mean ± SD or n (%) of participants.*

† Presence of cardiovascular diseases, i.e., coronary artery disease, stroke, congestive heart failure, arrhythmia, and peripheral arterial disease.

T0, gradual eGFR decline; T1, early non-decline and then persistent decline; T2, persistently increasing eGFR; T3, low baseline eGFR and then progressively increasing eGFR; MDRD, Modification of Diet in Renal Disease Study Equation for Estimating Glomerular Filtration Rate; BMI, body mass index; GNRI, Geriatric nutritional risk index; ACEI, Angiotensin-Converting-Enzyme Inhibitor; ARB, Angiotensin Receptor Blocker; OAD, oral antidiabetic.

and T3 groups were higher than those in the T0 and T1 groups. The median follow-up was 2.27 years [interquartile range (IQR), 1.16–4.45]. A total of 333 individuals (8.4%) died at the end of the study (**Table 1**).

Adjusted Associations of Trajectory Classes

Because the presence of certain parameters may be correlated with increased or decreased odds of trajectory classes (2), we then examined the association between the eGFR trajectory patterns and confounding factors via the multinomial logistic regression analysis (**Table 2**). After adjusting confounding factors, compared to the T0 group in the CKD cohort, eGFR trajectory T1 and T2 were associated with higher baseline eGFR values, but eGFR trajectory T3 was less likely to have high baseline eGFR. The estimated odds of being classified into the T1, T2, and T3 groups, rather than the T0 group, were 1.06 (95% CI 1.05–1.07), 1.13 (95% CI 1.11–1.14), and 0.96 (95% CI 0.95–0.97), respectively. Compared to the eGFR trajectory T0 group, the T1 group had higher serum albumin levels, and the T2 group had more elderly people, but the T3 group had a greater likelihood of hypertension. Compared to the T0 group, the eGFR trajectory T2 group had a higher proportion of older patients, while the eGFR trajectory T3 group had a higher proportion of younger patients. More specifically, for each additional year in age, there was a significant increase in the odds of being classified into the T2 group, but there was a decrease in the odds of being classified into the T3 group when compared to the T0 group.

TABLE 2 | Multinomial logistic regression to evaluate the association of patients' characteristics with different eGFR trajectory patterns.

TCVGH_CKD cohort	Gradual eGFR decline (T0)	Early non-decline and then persistent decline (T1)		Persistently increasing eGFR (T2)		Low baseline eGFR with the early decline and then progressively increasing eGFR (T3)	
	OR	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	1.0	1.00 (0.99–1.01)	0.95	1.09 (1.06–1.12)	<0.001	0.98 (0.97–1.00)	0.01
Male vs. female	1.0	1.27 (1.00–1.62)	0.05	0.90 (0.54–1.50)	0.69	0.91 (0.72–1.15)	0.42
Smoking	1.0	0.85 (0.67–1.09)	0.20	0.69 (0.42–1.14)	0.15	1.00 (0.77–1.29)	0.98
Alcohol	1.0	1.13 (0.88–1.44)	0.33	1.27 (0.75–2.14)	0.38	1.04 (0.81–1.34)	0.73
Diabetes mellitus	1.0	0.93 (0.77–1.13)	0.49	0.79 (0.52–1.22)	0.29	1.12 (0.93–1.36)	0.23
Hypertension	1.0	1.16 (0.94–1.42)	0.16	1.19 (0.78–1.82)	0.43	1.58 (1.28–1.95)	<0.001
Cardiovascular disease	1.0	1.07 (0.82–1.41)	0.60	1.54 (0.91–2.61)	0.11	0.83 (0.63–1.09)	0.18
Baseline eGFR	1.0	1.06 (1.05–1.07)	<0.001	1.13 (1.11–1.14)	<0.001	0.96 (0.95–0.97)	<0.001
Albumin	1.0	1.38 (1.13–1.69)	0.002	0.93 (0.64–1.33)	0.68	0.84 (0.69–1.02)	0.08
BMI	1.0	0.99 (0.96–1.03)	0.63	1.01 (0.96–1.07)	0.61	1.00 (0.96–1.03)	0.76

Applied multiple imputation.
OR, odds ratio; CI, confidence interval.

Clinical Characteristics Based on the Absence or Presence of Malnourishment

During the 12-year study period, 1,872 older adults had complete data with which to identify the status of malnourishment. Among these 1,872 patients, 765 (40.9%) were identified as having malnourishment. The study participants were predominantly male (66.7%), and the mean age was 76.9 years. The most common comorbidities were hypertension (77.7%) and DM (38.8%). Compared with the participants with malnourishment, those without malnourishment were younger, predominantly male, and more likely to have diabetes and/or hypertension. Participants without malnourishment also had higher alcohol consumption, HbA1c, and more prescriptions for hyperlipidemia and hypertension drugs (**Table 3**).

Correlations of eGFR Trajectory and Malnourishment With All-Cause Mortality and Kidney Failure

For all-cause mortality in the overall patients, compared to the T0 group, the eGFR trajectory T1 group had a lower risk (aHR = 0.52, 95% CI 0.32–0.83, $p = 0.007$) after adjusting for all other covariates (**Figure 3A**). More specifically, there was a lower risk for patients with malnourishment and eGFR trajectory T1 (aHR = 0.51, 95% CI 0.27–0.95, $p = 0.035$) (**Figure 3E**), but there was no significant risk for mortality in patients with eGFR trajectory T1 and without malnourishment (aHR = 0.52, 95% CI 0.25–1.08, $p = 0.08$) (**Figure 3C**).

Regarding the risk of kidney failure in the overall patients, eGFR trajectory T3 in patients significantly associated with a higher risk for kidney failure (aHR = 5.68, 95% CI 3.12–10.4, $p < 0.001$) compared to the reference (**Figure 3B**). In the presence of malnourishment and eGFR trajectory T3, the aHR was 5.85 (95% CI 2.37–14.58; **Figure 3F**); in patients with eGFR trajectory T3 and without malnourishment, the aHR was 5.49 (95% CI 2.45–12.27; **Figure 3D**) after compared with the reference.

Malnourishment and Comorbid Illness Are Risks for All-Cause Mortality and Kidney Failure

Concerning the effect of comorbid illness on all-cause mortality, baseline high eGFR and high GNRI value were significantly associated with a lower risk of death (**Figure 3A**) and kidney failure (**Figure 3B**), although there was no GNRI effect on kidney failure in patients with malnourishment (**Figure 3F**). In the subgroup analysis of eGFR on mortality, high eGFR was not significantly associated with all-cause mortality in the absence (**Figure 3C**) or presence (**Figure 3E**) of malnourishment. Patients of older age without or with malnourishment in the CKD cohort were associated with a high risk of death, respectively (without malnourishment: aHR = 1.09, 95% CI 1.05 −1.14, $p < 0.001$; **Figure 3C**; with malnourishment: aHR = 1.04, 95% CI 1.01–2.07, $p = 0.011$; **Figure 3E**). However, in the absence of malnourishment, patients with older age had a significantly lower risk of kidney failure than relatively young patients (**Figure 3D**). Moreover, DM in all patients (**Figure 3A**) and BMI > 24 in patients with malnourishment were significantly associated with a higher risk of death (**Figure 3E**). DM in all patients also had a higher risk of kidney failure (**Figure 3B**). Furthermore, patients with alcohol consumption had a high risk of kidney failure, especially those with malnourishment (**Figure 3F**). The multiplicative interaction analysis among eGFR trajectory, malnourishment, and comorbidities was significant in all-cause mortality as well as kidney failure. For mortality, patients with DM, eGFR trajectory T1, and BMI > 24 had different associations on the outcome between patients without malnourishment and patients with malnourishment. For kidney failure, patients with older age, alcohol consumption, and DM had different associations on the outcome between patients without and with malnourishment (**Supplementary Table 2**). The results of formal interaction analysis are almost consistent with those of the aforementioned analysis stratified by malnourishment. Furthermore, this association and predictive value of eGFR

TABLE 3 | Demographic and clinical characteristics between older adults without and with malnourishment.

Parameter*	Overall	Without malnourishment	With malnourishment	*p* value
Number of patients	***n* = 1,872**	***n* = 1,107**	***n* = 765**	
Demographic characteristics				
Age (years)	76.9 ± 6.7	76.5 ± 6.5	77.6 ± 6.9	0.001
Gender, male (%)	1,249 (66.7)	765 (69.1)	484 (63.3)	0.010
Smoking (%)	706 (37.9)	417 (37.7)	289 (37.8)	0.962
Alcohol (%)	493 (26.5)	318 (28.7)	175 (22.9)	0.006
Diabetes mellitus (%)	727 (38.8)	461 (41.6)	266 (34.8)	0.003
Hypertension (%)	1,454 (77.7)	918 (82.9)	536 (70.1)	<0.001
Cardiovascular diseases† (%)	224 (12.0)	125 (11.3)	99 (12.9)	0.313
Malignancy (%)	161 (9.1)	97 (8.8)	64 (8.4)	0.977
Baseline eGFR, ml/min per 1.73 m^2 by MDRD equation	31.1 ± 16.8	31.5 ± 15.4	30.5 ± 18.6	0.199
1. ≥90	13 (0.7)	5 (0.5)	8 (1.0)	0.216
2. 60–<90	82 (4.4)	40 (3.6)	42 (5.5)	
3. 30–<60	878 (46.9)	563 (50.9)	315 (41.2)	
4. 15–<30	557 (29.8)	321 (29.0)	236 (30.8)	
5. <15	342 (18.3)	178 (16.1)	164 (21.4)	
Malnourishment indicators				
Serum albumin <3.5g/dL, (%)	289 (15.4)	46 (4.2)	243 (31.8)	<0.001
BMI, kg/m^2	24.1 ± 3.7	26.1 ± 2.9	20.9 ± 2.5	<0.001
GNRI	104.9 ± 11.2	110.7 ± 7.0	93.5 ± 9.1	<0.001
eGFR trajectory (%)				0.988
T0	349 (18.6)	207 (18.7)	142 (18.6)	
T1	710 (37.9)	461 (41.6)	249 (32.5)	
T2	119 (6.4)	58 (5.2)	61 (8.0)	
T3	694 (37.1)	381 (34.4)	313 (40.9)	
Other laboratory data				
Serum uric acid ≥7.2 mg/dL (%)	917 (51.9)	644 (58.2)	327 (42.7)	0.331
HbA1c ≥6.5%, (%)	398 (21.3)	280 (25.3)	118 (15.4)	<0.001
Serum albumin, g/dL	3.9 ± 0.6	4.1 ± 0.3	3.5 ± 0.7	<0.001
Medications, n (%)				
ACEIs/ARBs	1,170 (62.5)	704 (63.6)	464 (60.6)	0.303
Insulin	622 (33.2)	340 (30.7)	288 (37.7)	0.105
OAD	667 (35.6)	436 (39.4)	231 (30.2)	<0.001
Antilipemic agents	472 (25.2)	294 (26.6)	152 (19.9)	0.004
Drugs for hypertension	1,380 (73.7)	808 (73.0)	495 (64.7)	0.005
Follow-up time, years, median (25th–75th percentile)	2.7 (1.3–4.3)	2.9 (1.4–4.3)	2.4 (1.2–4.3)	<0.001
Death (%)	139 (7.4)	57 (5.1)	82 (10.7)	<0.001

**Data are presented as mean ± SD or n (%) of participants.*

†Presence of cardiovascular diseases, i.e., coronary artery disease, stroke, congestive heart failure, arrhythmia, and peripheral arterial disease.

T0, gradual eGFR decline; T1, early non-decline and then persistent decline; T2, persistently increasing eGFR; T3, low baseline eGFR and then progressively increasing eGFR; MDRD, modification of diet in renal disease study equation for estimating glomerular filtration rate; BMI, body mass index; GNRI, Geriatric nutritional risk index; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker; OAD, oral antidiabetic.

trajectory T3 for all-cause mortality were inverse when the initial 3,948 older patients were without selection for malnourishment (**Table 4**).

DISCUSSION

To the best of our knowledge, this is the first study to characterize the full spectrum of different eGFR trajectory patterns in CKD older adults without or with malnourishment. We also found that longitudinal eGFR change was more useful than baseline eGFR or eGFR decline rate when the observation window was extended. This was tested and estimates for baseline GFR were statistical borderline in the models for both mortality and kidney failure (**Figure 3**). We characterized four phenotypically distinct functional trajectories. Although eGFR trajectory T0 and T1, respectively, showed a consistently slow decline of eGFR and early non-decline before persistent eGFR decline

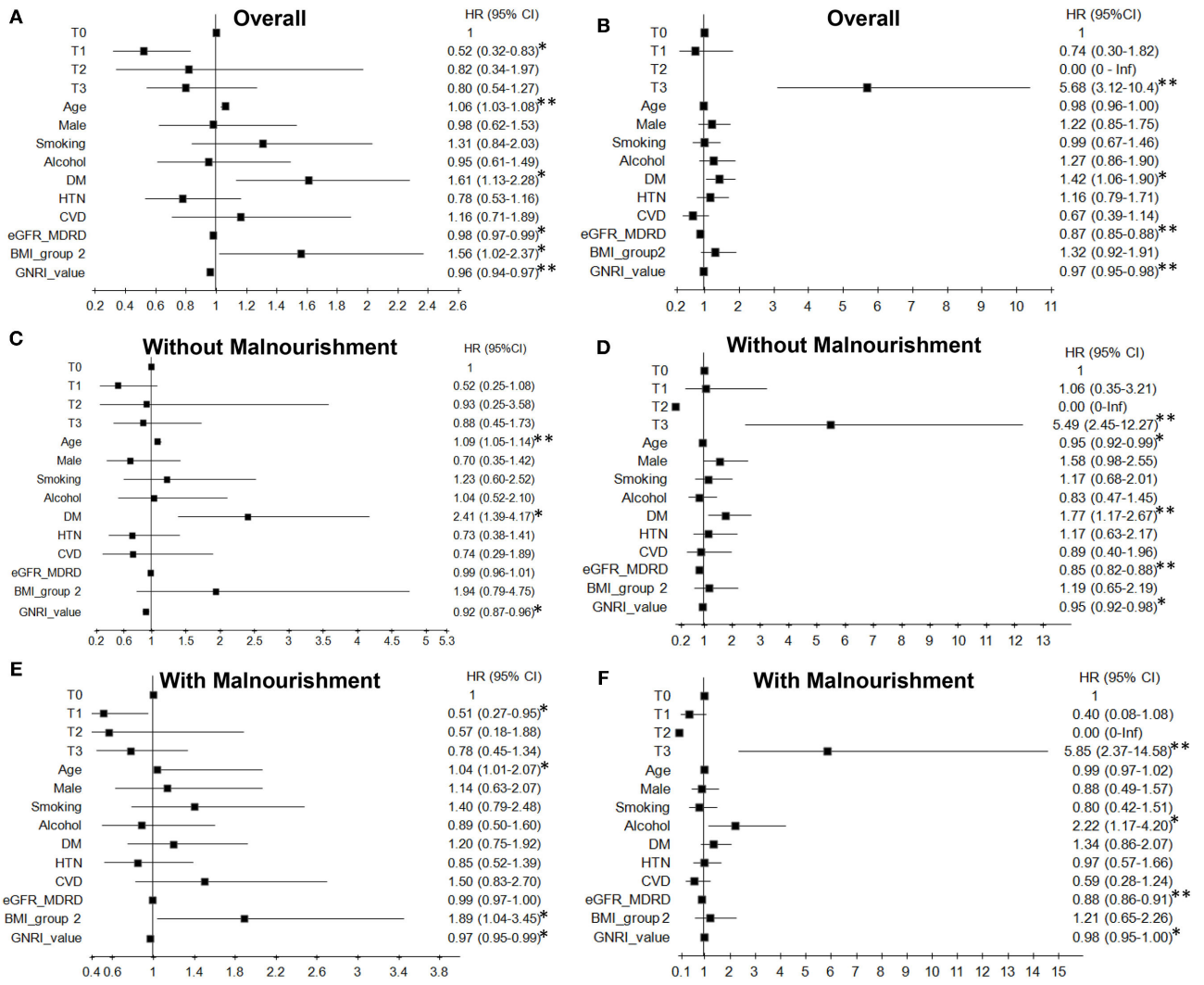


FIGURE 3 | Effects of kidney function trajectories and malnourishment on all-cause mortality **(A,C,E)** and kidney failure **(B,D,F)** among older adults. 1,872 older patients were investigated, and the outcomes were fully adjusted for demographics, laboratory parameters, clinical comorbid conditions, GNRI, and medication. There were no kidney failure events for the eGFR trajectory T2 pattern. HR, Hazard ratio; CI, confidence interval; DM, diabetes mellitus; HTN, hypertension; CVD, cardiovascular diseases; BMI_group 2, BMI cut-off point 24 kg/m^2. The normal BMI value: male is 19.2–23.7 kg/m^2 and female is 18.3–22.7 kg/m^2 (32). *$p < 0.05$, **$p < 0.001$.

in the CKD patients (1–3), trajectory T3, which showed low baseline eGFR with an early decline and increasing eGFR in the follow-up period, was notably and highly associated with kidney failure in older adults with malnourishment (**Figure 3F**). Besides, multimorbidity, abnormal BMI status, and malnutrition were significantly predictive of patients' outcomes in older adults (**Figure 3**).

The lower association of T1 with mortality is stated to be specifically present among patients with malnourishment. Although the estimate was not statistically significant for patients without malnourishment, it was nearly identical to estimates for the full cohort and the subset with malnourishment. The confounding factor due to malnourishment may specifically present to support the association of T1 with mortality. Those highlighted the intensive and integrated kidney disease care, including low protein diet, control of blood pressure, sugar, hyperlipidemia, correction of metabolic acidosis by sodium bicarbonate, and adequate administration of erythropoietin stimulating agents might slow the renal progression that is independently associated with lower risk for heart failure, myocardial infarction, and peripheral arterial disease (33, 34). Then, circulating the uremic milieu triggering chronic inflammation and oxidative stress-related cardiovascular events and all-cause death might be reduced, especially in the T1 group (35).

Similarly, the HRs for T3 and kidney failure was 5.85 and 5.49 for malnourished and not malnourished older patients, respectively. Both significant differences and with adequate

TABLE 4 | Effects of kidney function trajectories and comorbid illnesses on all-cause mortality among 3,948 older patients with chronic kidney disease without multiple imputation for missing data and without selection for malnourishment.

Parameters	TCVGH_CKD	
	HR (95% CI)*	*p* value
eGFR trajectory		
T0 (gradual eGFR decline)	1.0 –	–
T1 (early non-decline and then persistent decline)	0.46 (0.35–0.62)	<0.001
T2 (persistently increasing eGFR)	0.37 (0.17–0.83)	0.02
T3 (low baseline eGFR with early decline and then progressively increasing eGFR)	0.70 (0.54–0.92)	0.009
Age	1.08 (1.06–1.09)	<0.001
Male	1.16 (0.87–1.56)	0.32
Smoking	1.09 (0.81–1.47)	0.57
Alcohol	0.94 (0.68–1.30)	0.70
DM	1.23 (0.97–1.55)	0.09
HTN	0.56 (0.44–0.72)	<0.001
CVD	0.99 (0.98–1.39)	0.94
Baseline eGFR	0.99 (0.98–0.99)	0.002
BMI_group 2	1.16 (0.82–1.65)	0.40
GNRI_value	0.97 (0.96–0.98)	<0.001

**Fully adjusted for demographics, laboratory parameters, clinical comorbid conditions, GNRI, and medication.*

HR, hazard ratio; CI, confidence interval; DM, diabetes mellitus; HTN, hypertension; CVD, cardiovascular diseases; BMI_group 2, BMI cut-off point 24 kg/m^2.

The normal BMI value: male is 19.2–23.7 kg/m^2 and female is 18.3–22.7 kg/m^2 (32).

confidence intervals claim that the HR decreased from one group to the other. Those may suggest in the data that these associations meaningfully differ, and again, the determinants might be presented.

Several potential mechanisms may explain the determinant of malnourishment on eGFR trajectory T3-associated kidney failure. First, malnutrition harming age-related multimorbidity, subsequent sarcopenia, and neuroendocrine dysfunction eventually results in an early decreasing and then increasing eGFR, and the vicious cycle also negatively impacts survival and renal outcome (36). Second, in studies conducted by the US Department of Veterans Affairs (VA), 66% of patients died without having composite kidney disease and 34% of patients died after developing kidney disease (2, 10). Most affected patients were older adults. It is plausible that we speculated that the eGFR trajectory patterns and selection of malnourishment would simultaneously involve multimorbidity and nutritional status, and our analysis indeed showed that these factors were correlated with mortality or kidney failure in older adults. Finally, the underlying effect of malnourishment on kidney failure in older people with increasing eGFR in the follow-up period may include undernourishment, sarcopenic obesity (BMI > 24), and multimorbidity rather than underweight status, which can lead to dysregulation or decompensation of renal function (4). This finding is also compatible with low creatinine appearance in kidney disease cachexia in the criteria of low muscle mass, especially in older adults (37).

Initial low eGFR has been attributed to several concordant and discordant comorbid conditions, which had direct or indirect associations with the outcome (2). Baseline malnourishment status may play a prominent role in the mechanisms underlying the effect of increasing eGFR trajectory patterns on kidney failure before the death event in older people. We used baseline eGFR and eGFR trajectory patterns to test the outcomes and found high or low eGFR value presented a considerable clinical challenge, which may frustrate attempts to achieve optimal outcomes in patients with coexisting diseases.

A major strength of this study was the differences in mortality rates and kidney failure among four eGFR trajectory patterns which have not been previously done in the literature. Secondly, we showed that the most striking finding in the present study was the association of eGFR trajectory and comorbid conditions with the risk of outcomes which is modified by malnourishment status. It is not inferior to the diagnostic performance of several available tests, such as integrated discrimination index (IDI) or net reclassification improvement (27, 38) for the candidate biomarkers (longitudinal measurements of cystatin C) in terms of accuracy of risk prediction (6). Thirdly, our method of analysis was capable of fully interpreting the nonlinear eGFR trajectories or a prolonged period of non-progression in intrinsic or extrinsic renal diseases.

There were some potential limitations in the present study that should be addressed. Firstly, there were not enough variables for comparison due to the large size of the cohort. Other possible confounding factors included several concordant and discordant comorbid conditions. Since albuminuria and proteinuria were not included in our dataset, the accuracy of nutritional indicators such as albumin and GNRI is questionable. Especially in patients with nephrotic proteinuria, these nutritional indicators may not adequately reflect nutritional status. Secondly, this study included mostly Taiwanese individuals with a highly selected population, and thus the results may not be generalizable to less narrowly defined populations. Thirdly, old age seemed to have protective effects on kidney failure in the CKD cohort, and this finding was consistent with previous findings. The age distribution was the same between the results obtained using multiple imputations and those without any imputations (**Supplementary Figure 2**). Fourth, in clinical practice, it is necessary to early diagnose the risk of nutritional disorders and kidney failure for starting treatment promptly and preventing their progression. However, it takes years to diagnose which of the four eGFR trajectory patterns a patient falls into. Fifth, the initiation, dose increase, and discontinuation of drugs that affect glomerular filtration such as angiotensin-converting-enzyme inhibitor and angiotensin receptor blocker may influence the eGFR trajectory pattern. However, it is difficult to present in this study.

In conclusion, eGFR trajectories were shown to be a valuable prognostic indicator for predicting outcomes in older adults with CKD. An integrated kidney disease care program could have a notable beneficial effect on patients'

mortality and kidney failure, based on a comparison with gradual eGFR decline. Increasing eGFR trajectory in the later period was shown to be a high-risk factor for kidney failure in older CKD patients. These phenomena may be due to multimorbidity, abnormal BMI status, and malnutrition.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Taichung Veterans General Hospital (Nos. CF13015, CF13015-1, CF13015-2, CF13015-3, and CE12252-1). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

S-CW wrote the manuscript. C-MC, Y-CC, M-JW, and D-CT conceived and designed the experiments and contributed to the discussion and manuscript revision. C-MC performed the analyses. S-CW, M-JW, and D-CT performed the experiments and collected the data. M-JW and D-CT conceived the study and are the guarantors of this publication. All authors reviewed the manuscript, contributed to the article, and approved the submitted version.

FUNDING

We are also deeply indebted to Taichung Veterans General Hospital, Taichung for providing the grants for this study (TCVGH-YM1050101, TCVGH-1068201B, TCVGH-YM1060103, TCVGH-1078201B, TCVGH-YM1070101, TCVGH-1088201B, TCVGH-YM1080103, TCVGH-1098201B, TCVGH-1108201B, TCVGH-1108202D, and TCVGH-YM1090105). This study was also supported by Taiwan's Ministry of Science and Technology (MOST 106-2314-B-075A-003) and the Center for Intelligent Drug Systems and Smart Bio-devices (IDS2B) from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan and the Foundation for Poison Control.

ACKNOWLEDGMENTS

We are grateful to the Biostatistics Task Force of Taichung Veterans General Hospital, Taichung, Taiwan, ROC, for assistance in statistics. This study was also supported by Rong Sing Medical Foundation. The authors sincerely appreciate the assistance of the Center for Translational Medicine of Taichung Veterans General Hospital, Taichung, Taiwan. Patient advisers were also credited with the contributions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.760391/full#supplementary-material

REFERENCES

1. Li L, Astor BC, Lewis J, Hu B, Appel LJ, Lipkowitz MS, et al. Longitudinal progression trajectory of GFR among patients with CKD. *Am J Kidney Dis.* (2012) 59:504–12. doi: 10.1053/j.ajkd.2011.12.009
2. Xie Y, Bowe B, Xian H, Balasubramanian S, Al-Aly Z. Estimated GFR trajectories of people entering CKD stage 4 and subsequent kidney disease outcomes and mortality. *Am J Kidney Dis.* (2016) 68:219–28. doi: 10.1053/j.ajkd.2016.02.039
3. O'Hare AM, Batten A, Burrows NR, Pavkov ME, Taylor L, Gupta I, et al. Trajectories of kidney function decline in the 2 years before initiation of long-term dialysis. *Am J Kidney Dis.* (2012) 59:513–22. doi: 10.1053/j.ajkd.2011.11.044
4. Ou SM, Chen YT, Hung SC, Shih CJ, Lin CH, Chiang CK, et al. Association of estimated glomerular filtration rate with all-cause and cardiovascular mortality: the role of malnutrition-inflammation-cachexia syndrome. *J Cachexia Sarcopenia Muscle.* (2016) 7:144–51. doi: 10.1002/jcsm.12053
5. Chen YC, Weng SC, Liu JS, Chuang HL, Hsu CC, Tarng DC. Severe decline of estimated glomerular filtration rate associates with progressive cognitive deterioration in the elderly: a community-based cohort study. *Sci Rep.* (2017) 7:42690. doi: 10.1038/srep42690
6. Rifkin DE, Shlipak MG, Katz R, Fried LF, Siscovick D, Chonchol M, et al. Rapid kidney function decline and mortality risk in older adults. *Arch Intern Med.* (2008) 168:2212–8. doi: 10.1001/archinte.168.20.2212
7. Shlipak MG, Katz R, Kestenbaum B, Siscovick D, Fried L, Newman A, et al. Rapid decline of kidney function increases cardiovascular risk in the elderly. *J Am Soc Nephrol.* (2009) 20:2625–30. doi: 10.1681/ASN.2009050546
8. Matsushita K, Selvin E, Bash LD, Franceschini N, Astor BC, Coresh J. Change in estimated GFR associates with coronary heart disease and mortality. *J Am Soc Nephrol.* (2009) 20:2617–24. doi: 10.1681/ASN.2009010025
9. Al-Aly Z, Zeringue A, Fu J, Rauchman MI, McDonald JR, El-Achkar TM, et al. Rate of kidney function decline associates with mortality. *J Am Soc Nephrol.* (2010) 21:1961–9. doi: 10.1681/ASN.2009121210
10. Al-Aly Z, Balasubramanian S, McDonald JR, Scherrer JF, O'Hare AM. Greater variability in kidney function is associated with an increased risk of death. *Kidney Int.* (2012) 82:1208–14. doi: 10.1038/ki.2012.276
11. Coresh J, Turin TC, Matsushita K, Sang Y, Ballew SH, Appel LJ, et al. Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. *JAMA.* (2014) 311:2518–31. doi: 10.1001/jama.2014.6634
12. Jiang G, Luk AOY, Tam CHT, Xie F, Carstensen B, Lau ESH et al. Progression of diabetic kidney disease and trajectory of kidney function decline in Chinese patients with Type 2 diabetes. *Kidney Int.* (2019) 95:178–87. doi: 10.1016/j.kint.2018.08.026
13. Cox HJ, Bhandari S, Rigby AS, Kilpatrick ES. Mortality at low and high estimated glomerular filtration rate values: a 'U' shaped curve. *Nephron Clin Pract.* (2008) 110:c67–72. doi: 10.1159/000151720
14. Anand N. S C C, Alam MN. The malnutrition inflammation complex syndrome-the micsing factor in the perio-chronic kidney disease interlink. *J Clin Diagn Res.* (2013) 7:763–7. doi: 10.7860/JCDR/2013/5329.2907
15. Landi F, Calvani R, Tosato M, Martone AM, Bernabei R, Onder G, et al. Impact of physical function impairment and multimorbidity on mortality among community-living older persons with sarcopaenia: results from

the ilSIRENTE prospective cohort study. *BMJ Open.* (2016) 6:e008281. doi: 10.1136/bmjopen-2015-008281
16. Lin TY, Peng CH, Hung SC, Tarng DC. Body composition is associated with clinical outcomes in patients with non-dialysis-dependent chronic kidney disease. *Kidney Int.* (2018) 93:733–40. doi: 10.1016/j.kint.2017.08.025
17. Sharma D, Hawkins M, Abramowitz MK. Association of sarcopenia with eGFR and misclassification of obesity in adults with CKD in the United States. *Clin J Am Soc Nephrol.* (2014) 9:2079–88. doi: 10.2215/CJN.02140214
18. Wu CY, Chou YC, Huang N, Chou YJ, Hu HY Li CP. Association of body mass index with all-cause and cardiovascular disease mortality in the elderly. *PLoS ONE.* (2014) 9:e102589. doi: 10.1371/journal.pone.0102589
19. Weng SC, Tarng DC, Chen CM, Cheng CH, Wu MJ, Chen CH, et al. Estimated glomerular filtration rate decline is a better risk factor for outcomes of systemic disease-related nephropathy than for outcomes of primary renal diseases. *PLoS ONE.* (2014) 9:e92881. doi: 10.1371/journal.pone.0092881
20. Weaver RG, James MT, Ravani P, Weaver CGW, Lamb EJ, Tonelli M, et al. Estimating urine albumin-to-creatinine ratio from protein-to-creatinine ratio: development of equations using same-day measurements. *J Am Soc Nephrol.* (2020) 31:591–601. doi: 10.1681/ASN.2019060605
21. Kamińska J, Dymicka-Piekarska V, Tomaszewska J, Matowicka-Karna J, Koper-Lenkiewicz OM. Diagnostic utility of protein to creatinine ratio (P/C ratio) in spot urine sample within routine clinical practice. *Crit Rev Clin Lab Sci.* (2020) 57:345–64. doi: 10.1080/10408363.2020.1723487
22. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, et al. more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
23. Cheng HT, Huang JW, Chiang CK, Yen CJ, Hung KY, Wu KD. Metabolic syndrome and insulin resistance as risk factors for development of chronic kidney disease and rapid decline in renal function in elderly. *J Clin Endocrinol Metab.* (2012) 97:1268–76. doi: 10.1210/jc.2011-2658
24. Pinheiro JC, Bates DM. *Mixed-Effects Models in S and S-PLUS*. New York, NY: Springer. (2002). doi: 10.1007/978-1-4419-0318-1
25. Jocelyn W, Sanjeevkumar RP. *Changes in Kidney Function. Hazzard's Geriatric Medicine and Gerontology*. 6th edn. Seattle: McGraw-Hill (2009), p. 1009–15.
26. Lu TFC, Hsu CM, Shu KH, Weng SC, Chen CM. Joint analysis of longitudinal data and competing terminal events in the presence of dependent observational times: application to chronic kidney disease. *J Appl Stat.* (2016) 43:2922–40. doi: 10.1080/02664763.2016.1155202
27. Matsushita K, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, et al. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA.* (2012) 307:1941–51. doi: 10.1001/jama.2012.3954
28. Bouillanne O, Morineau G, Dupont C, Coulombel I, Vincent JP, Nicolis I, et al. Geriatric Nutritional Risk Index: a new index for evaluating at-risk elderly medical patients. *Am J Clin Nutr.* (2005) 82:777–83. doi: 10.1093/ajcn/82.4.777
29. Yamada K, Furuya R, Takita T, Maruyama Y, Yamaguchi Y, Ohkawa S, et al. Simplified nutritional screening tools for patients on maintenance hemodialysis. *Am J Clin Nutr.* (2008) 87:106–13. doi: 10.1093/ajcn/87.1.106
30. Tsai MT, Hu FH, Lien TJ, Chen PJ, Huang TP, Tarng DC. Interaction between geriatric nutritional risk index and decoy receptor 3 predicts mortality in chronic hemodialysis patients. *Am J Nephrol.* (2014) 40:191–9. doi: 10.1159/000366457
31. Liu Y, De A. Multiple imputation by fully conditional specification for dealing with missing data in a large epidemiologic study. *Int J Stat Med Res.* (2015) 4:287–95. doi: 10.6000/1929-6029.2015.04.03.7
32. Department of Health, Executive Yuan. *Spindle 2009 Health Education Advocacy Plan Survey Summary Report—The Definition of Body Mass Index in Adults in Taiwan*, *Vol* 36. Taiwan: Department of Health (2009), p. 23–6.
33. Tsai CW, Ting IW, Yeh HC, Kuo CC. Longitudinal change in estimated GFR among CKD patients: a 10-year follow-up study of an integrated kidney disease care program in Taiwan. *PLoS ONE.* (2017) 12:e0173843. eCollection 2017. doi: 10.1371/journal.pone.0173843
34. Kim HJ Ryu H, Kang E, Kang M, Han M, Song SH, et al. Metabolic acidosis is an independent risk factor of renal progression in Korean chronic kidney disease patients: the KNOW-CKD Study results. *Front Med.* (2021) 8:707588. eCollection 2021. doi: 10.3389/fmed.2021.707588
35. Chang JF, Liu SH, Lu KC, Ka SM, Hsieh CY, Ho CT, et al. Uremic vascular calcification is correlated with oxidative elastic lamina injury, contractile smooth muscle cell loss, osteogenesis, and apoptosis: the human pathobiological evidence. *Front Med.* (2020) 7:78. eCollection 2020. doi: 10.3389/fmed.2020.00078
36. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci.* (2001) 56:M146–56. doi: 10.1093/gerona/56.3.m146
37. Koppe L, Fouque D, Kalantar-Zadeh K. Kidney cachexia or protein-energy wasting in chronic kidney disease: facts and numbers. *J Cachexia Sarcopenia Muscle.* (2019) 10:479–84. doi: 10.1002/jcsm.12421
38. Pickering JW, Endre ZH. New metrics for assessing diagnostic potential of candidate biomarkers. *Clin J Am Soc Nephrol.* (2012) 7:1355–64. doi: 10.2215/CJN.09590911

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

MINI REVIEW
published: 07 December 2021
doi: 10.3389/fmed.2021.790513

Non-classical Vitamin D Actions for Renal Protection

Adriana S. Dusso[1]*, *Kevin T. Bauerle*[1,2] and *Carlos Bernal-Mizrachi*[1,2,3]

[1] Division of Endocrinology, Metabolism and Lipid Research, Department of Medicine, Washington University School of Medicine, St. Louis, MO, United States, [2] Department of Medicine, VA Medical Center, St. Louis, MO, United States, [3] Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO, United States

Chronic Kidney Disease (CKD), a disorder that affects 11% of the world's population, is characterized by an acceleration in skeletal, immune, renal, and cardiovascular aging that increases the risk of cardiovascular mortality by 10- to 20-fold, compared to that in individuals with normal renal function. For more than two decades, the progressive impairment in renal capacity to maintain normal circulating levels of the hormonal form of vitamin D (1,25-dihydroxyvitamin D or calcitriol) was considered the main contributor to the reduced survival of CKD patients. Accordingly, calcitriol administration was the treatment of choice to attenuate the progression of secondary hyperparathyroidism (SHPT) and its adverse impact on bone health and vascular calcification. The development of calcitriol analogs, designed to mitigate the resistance to calcitriol suppression of PTH associated with CKD progression, demonstrated survival benefits unrelated to the control of SHPT or skeletal health. The exhaustive search for the pathophysiology behind survival benefits associated with active vitamin D analogs has identified novel anti-inflammatory, anti-hypertensive, anti-aging actions of the vitamin D endocrine system. A major paradigm shift regarding the use of calcitriol or active vitamin D analogs to improve survival in CKD patients emerged upon demonstration of a high prevalence of vitamin D (not calcitriol) deficiency at all stages of CKD and, more significantly, that maintaining serum levels of the calcitriol precursor, 25(OH)vitamin D, above 23 ng/ml delayed CKD progression. The cause of vitamin D deficiency in CKD, however, is unclear since vitamin D bioactivation to 25(OH)D occurs mostly at the liver. Importantly, neither calcitriol nor its analogs can correct vitamin D deficiency. The goals of this chapter are to present our current understanding of the pathogenesis of vitamin D deficiency in CKD and of the causal link between defective vitamin D bioactivation to calcitriol and the onset of molecular pathways that promote CKD progression independently of the degree of SHPT. An understanding of these mechanisms will highlight the need for identification of novel sensitive biomarkers to assess the efficacy of interventions with vitamin D and/or calcitriol(analogs) to ameliorate CKD progression in a PTH-independent manner.

Keywords: klotho, ADAM17, FGF23, hypertension, ACE2, systemic inflammation, chronic kidney disease

OPEN ACCESS

Edited by:
Ana Cusumano, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Argentina

Reviewed by:
Mario Cozzolino, University of Milan, Italy
Jose L. Mansur, National University of La Plata, Argentina

***Correspondence:**
Adriana S. Dusso
adusso@wustl.edu

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *06 October 2021*
Accepted: *15 November 2021*
Published: *07 December 2021*

Citation:
Dusso AS, Bauerle KT and Bernal-Mizrachi C (2021) Non-classical Vitamin D Actions for Renal Protection. Front. Med. 8:790513. doi: 10.3389/fmed.2021.790513

INTRODUCTION

The vitamin D endocrine system is critical for human health, and a structurally normal kidney is essential to maintain the functional integrity of the vitamin D endocrine system. In the general population, vitamin D deficiency is associated with an increased relative risk for cardiovascular and all-cause mortality (1, 2). Accordingly, in chronic kidney disease (CKD), a disorder that affects 11% of the world's population, there are progressive reductions in renal capacity to produce the active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol), and a surprising inability to maintain circulating vitamin D levels, resulting in an accelerated immune, skeletal, renal and cardiovascular aging. The end-result is a 10–20-fold increase in cardiovascular morbidity and mortality, compared to those in gender- and age-matched individuals with normal renal function (3, 4).

Defects in mineral metabolism, including intestinal calcium absorption and renal phosphate excretion, are best characterized features of CKD associated with vitamin D/calcitriol deficiency. These defects in mineral metabolism contribute to the onset and progression of secondary hyperparathyroidism (SHPT) and its associated abnormal bone remodeling including defective mineralization, increases in bone resorption and fracture risk and the propensity to vascular and soft tissue calcifications (5). It is also important to note that hyperphosphatemia is negatively correlated with lifespan in mammals. Maintenance of phosphate homeostasis requires a complex endocrine network involving PTH, vitamin D, FGF 23, and Klotho to provide appropriate signals to the kidney, parathyroid glands, gut, and bone. While hyperphosphatemia is known to stimulate pro-inflammatory, pro-aging and pro-fibrotic signals to exacerbate renal and cardiovascular damage (6), the mechanisms by which active vitamin D induces expression of the *FGF23* and *α-klotho* genes to attenuate the pro-aging effects of hyperphosphatemia and maintain the plethora of anti-aging/pro-survival actions of renal and circulating klotho are not fully understood (7).

The prevalence of vitamin D deficiency increases with advancing CKD (8) and has a more severe impact on survival than that of calcitriol deficiency (9). This finding has challenged 30 years of clinical experience, where calcitriol was exclusively administered to control SHPT. In fact, particularly pertinent to topic of this review on vitamin D prevention of CKD progression, levels of the calcitriol precursor 25(OH)D above 23 ng/ml and not of calcitriol are independently associated with reno-protective actions (10). Since neither calcitriol nor its analogs correct vitamin D deficiency, and in view of the controversies from prospective trials regarding the actual efficacy of vitamin D supplementation strategies to improve survival in individuals with normal renal function, or SHPT and kidney injury in early CKD, the overall goal of this chapter is to update our understanding on (1) CKD-induced defects in systemic and local vitamin D bioactivation and calcitriol actions and (2) outline approaches to improve strategies to effectively ameliorate CKD progression independently of the degree of SHPT by attenuating its strongest inducers, systemic inflammation, hypertension, renal and cardiovascular damage. Special focus will be directed at the pathophysiology driving calcitriol/VDR-mediated reduction in pro-inflammatory and hypertensive signals unrelated to klotho reductions that promote multi-organ damage, including:

1) Hypertension-driven renal and vascular damage unrelated to the suppression of renin gene expression
2) Immune cell-driven systemic inflammation and oxidative stress-mediated multi-organ damage
3) Inflammation-induced renin-driven hypertension.

This mechanistic knowledge is a mandatory first step to evaluate the accuracy of current biomarkers of the severity of CKD progression and of the response to vitamin D therapy.

DEFECTIVE VITAMIN D BIOACTIVATION TO CALCITRIOL IN CKD

Vitamin D is not truly a vitamin because, in mammals, the sun light [ultraviolet B range (UV-B)] converts the skin precursor 7-dehydrocholesterol into vitamin D_3 (cholecalciferol) (11). This conversion is completely prevented by sunscreen (12). The next step occurs primarily in the liver, where two cytochromes p450 enzymes, mitochondrial CYP27A1 and microsomal CYP2R1, hydroxylate cholecalciferol at the 25-position to produce 25-hydroxyvitamin D (25(OH)D), the main circulating vitamin D metabolite, with a biological half- life of 15–18 days. Since only mutations in CYP2R1 result in severe vitamin D deficiency (13) (serum 25(OH)D levels below 10 ng/ml), CYP2R1 is considered the most critical vitamin D-25-hydroxylase. Even though skin and parathyroid cells, immune cells and endometrial cells express 25-hydroxylases (14, 15), neither their distribution in other tissues nor the regulation of their expression or activity are fully understood. Nevertheless, because vitamin D measurements require assays too complex for routine biochemistry laboratories, and 25-hydroxylases efficaciously convert most circulating vitamin D into 25(OH)D (16), measurements of 25(OH)D levels are used to estimate the vitamin D status of an individual.

An important clinical consideration is that only mass spectrometry accurately measures 25(OH)D levels. Most common assays used worldwide have a 100% cross reactivity of 25(OH)D with 24,25$(OH)_2$D, a metabolite that marks the first step in 25(OH)D degradation, and consequently, there is an overestimation of vitamin D status.

The most critical and tightly regulated step in vitamin D bioactivation occurs mainly, although not exclusively, in renal proximal tubules, where the mitochondrial cytochrome p450 CYP27B1 catalyzes the 1α-hydroxylation of 25(OH)D to produce calcitriol, the most active endogenous vitamin D metabolite (17). Under physiological conditions, this renal conversion is the main, if not the only, contributor to circulating calcitriol. Thus, vitamin D is actually an inactive pro-hormone whose biological actions require a two-step bioactivation to 1,25-dihydroxyvitaminD (1,25D or calcitriol), a potent steroid hormone (Depicted in **Figure 1**) (11).

In contrast to the 25-hydroxylases, where the enzymatic activity is not closely regulated, CYP27B1 is tightly regulated to maintain serum calcitriol within the narrow limits required to avoid hypercalcemic and hyper-phosphatemic episodes.

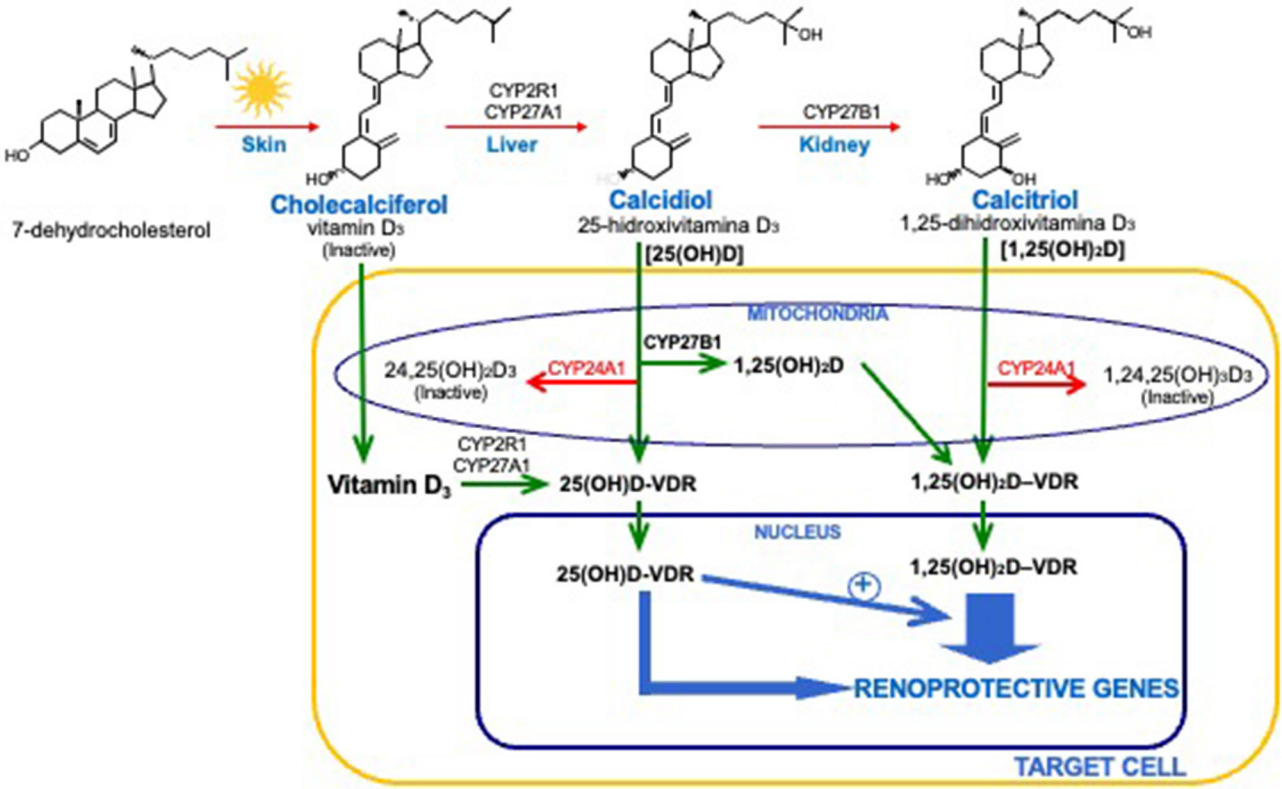


FIGURE 1 | Vitamin D Bioactivation and calcitriol/VDR renoprotective actions. Systemic and intracellular vitamin D activation to 25(OH)D and calcitriol; intracellular balance between activating CYP2R1/CYP27A1 or CYP27B1 with catabolizing CYP24A1 responsible for final 25(OH)D and calcitriol concentrations for VDR binding and activation, and 25(OH)/calcitriol-synergy to enhance calcitriol-VDR transcriptional regulation of renoprotective genes.

CYP27B1 is induced by PTH and suppressed by FGF23 and klotho. Calcitriol itself also controls its own circulating levels through a feed-back suppression of its own synthesis, but mostly through the induction of CYP24A1 gene expression. This enzyme, responsible for calcitriol and 25(OH)D degradation, is constitutively expressed in the kidney and strongly induced by either calcitriol or its analogs in every vitamin D target tissue, thus reducing the toxicity associated with high circulating calcitriol (18). Accordingly, to maintain normal circulating calcitriol levels, PTH and FGF23 suppress and induce CYP24A1, respectively.

Numerous other cell types express CYP27B1 and produce calcitriol in a cell/tissue specific manner. This extrarenal calcitriol production is of high clinical relevance beyond CKD. In fact, the multiple health disorders associated to vitamin D deficiency in the general population occur despite normal serum calcitriol. The low circulating 25(OH)D levels during vitamin D deficiency (below 20 ng/ml) reduce local calcitriol synthesis by non-renal cyp27B1s, thus compromising the plethora of tissue-specific pro-survival actions of calcitriol.

CKD severely compromises renal and extrarenal vitamin D bioactivation to calcitriol starting with an impaired photo-activation of the skin precursor 7-dehydrocholesterol to cholecalciferol (19).

The high incidence of vitamin D deficiency at all stages of CKD (8) emphasizes the critical role of normal kidney function in maintaining normal serum 25(OH)D by facilitating the reabsorption of 25(OH)D that has been previously filtered at the glomerulus. 25(OH)D is a lipid soluble molecule that circulates bound to its main carrier, the vitamin D binding protein (DBP), a low molecular weight protein (60 KDaltons), similar in mass to albumin. The 25(OH)D/DBP complex is filtered at the glomerulus and its reabsorption occurs at proximal tubules and requires adequate tubular cell levels of the endocytosis receptor, megalin (20). In CKD, an early loss of renal megalin (21) impairs the renal uptake of 25(OH)D compromising non only mitochondrial calcitriol production, but also 25(OH)D recycling back to the circulation to ensure adequate extrarenal calcitriol synthesis. Interestingly, vitamin D induces the expression of renal megalin (22). Thus, the early correction of vitamin D deficiency in CKD recommended by KDIGO guidelines may attenuate renal megalin reductions, thereby improving renal and extrarenal calcitriol production.

The actual contribution of impaired uptake of 25(OH)D on renal calcitriol production was demonstrated by a strong correlation between serum calcitriol and 25(OH)D levels in advanced CKD patients with a glomerular filtration rate below 25 ml/min (23, 24), which does not exist in individuals with

normal renal function. Importantly, in addition to defective substrate availability for calcitriol production in CKD, a higher fragmentation of PTH in the parathyroid gland, elevations in FGF23, and the accumulation of uremic toxins further reduce CYP27B1 expression to exacerbate calcitriol deficiency, as reviewed in (17).

Importantly, 25(OH)D supplementation to hemodialysis patients can normalize serum calcitriol, even though renal mRNA levels for CYP27B1 should be markedly reduced by high serum FGF23 (24). The contribution of extrarenal calcitriol production cannot be fully disregarded as FGF23 increases rather than decreases parathyroid CYP27B1 expression (25). Similarly, despite the increases in CYP24A1 mRNA induced by high FGF23, serum levels of 24,25-dihydroxyvitamin D in non-supplemented or in cholecalciferol supplemented patients were persistently lower than normal (26, 27). Clearly, in advanced CKD, the activity of either enzyme fails to reflect FGF23 control of the respective genes—that is, the damaged kidney fails to respond to regulation of renal calcitriol production by FGF23.

Furthermore, as will be discussed in the section of vitamin D control of systemic inflammation, CKD also impairs the substrate availability for immune cell calcitriol production.

DEFECTIVE CALCITRIOL-VITAMIN D RECEPTOR (VDR) ACTIONS IN CKD

Once calcitriol is synthesized in the kidney or extrarenally, most of its biological actions are mediated by binding to the VDR. VDR binding of calcitriol promotes a conformational change in VDR that facilitates heterodimerization with the retinoid X receptor (RXR) and the binding of the VDR/RXR complex to vitamin D responsive sequences (VDREs) in the promoter regions of vitamin D responsive genes to regulate gene expression (18). There are multiple simultaneous rather than a single site for binding of the ligand-activated VDR/RXR complex up or downstream from the transcription start site of a target gene (28) which are juxtaposed by chromatin looping, thus facilitating the recruitment of basic transcription factors, co-activator and/or co-repressor molecules to multiply the potency of VDR-RXR/VDRE complexes at regulating vitamin D responsive genes (29).

These calcitriol/VDR-DNA interactions activate/repress the expression of the 500–1,000 genes responsible for the survival benefits associated with normal vitamin D status. The most well-characterized calcitriol/VDR actions in nephrology relate to the control of the calcium/PTH and phosphate/FGF23-klotho axes, including the suppression of PTH synthesis and the induction of the phosphaturic hormone FGF23, the longevity gene klotho, and the calcium channel TRPV6 in enterocytes, the rate limiting step in intestinal calcium absorption. Other upregulated genes include the parathyroid calcium sensing receptor, which controls the responsiveness of the parathyroid gland to serum calcium and senses high circulating phosphate suppressing its inhibitory effect on PTH (30), and the receptor of the canonical Wnt pathway LRP5 in bone (18).

Some of these calcitriol/VDR target genes code for microRNAs (31), short (18–25 nucleotides) non-coding RNAs that control the expression of more than 30% of the genes in the genome by binding the 3' untranslated region of target mRNA, decreasing either their stability or protein translation. Calcitriol/VDR induction of miR-145 is of particular interest. miR-145 is the most abundant microRNA in normal vascular smooth muscle cells and may contribute to vascular protection, as miR145 is downregulated in proliferative vascular diseases (32) and also in uremia and hyperphosphatemia (33, 34). Reductions in miR145 directly augment ADAM17 gene expression, an enzyme which is critical for (1) the severity of parathyroid hyperplasia in the course of CKD (35), (2) release of TNFα that enhances systemic inflammation and multiorgan damage (36), and (3) angiotensin II-driven renal damage at early CKD stages (37).

The calcitriol/VDR complex also indirectly controls certain "apparent" target genes through the regulation of the expression of an essential inducer or repressor gene. Important examples related to attenuation of CKD progression include induction of C/EBPβ to facilitate suppression of parathyroid ADAM17 gene expression, which is critical to attenuate hyperplastic growth of parathyroid tissue (38). Additionally, VDR activation results in a 30-fold induction of FGF23 expression, critical for the renal handling of excess phosphate, which is markedly attenuated by inhibiting new protein synthesis. This finding suggests that the increase in FGF23 expression by calcitriol/VDR occurs via a yet unknown mediator (39).

Undoubtedly, the intracellular levels of both calcitriol and VDR determine the magnitude of calcitriol/VDR complex formation and the efficacy for direct or indirect control of target genes expression by the calcitriol/VDR complex, and both are reduced in CKD (17). Decreases in cellular levels of the VDR partner for the regulation of gene expression, the retinoid X receptor (RXR), as well as increases in systemic levels of uremic toxins reduce calcitriol/VDR-RXR binding to DNA, further impairing the response of CKD patients to vitamin D therapy [Reviewed in (40)].

Therapeutically, the development of calcitriol analogs that selectively maintain the benefits of VDR activation with less calcemic or phosphatemic activity (41) has helped improve outcomes, by allowing a safer escalation of analog dosage to counteract the progressive resistance to therapy caused by CKD-induced VDR reductions (42). Two of these "so called" analogs, 1-α-hydroxyvitamin D3 and 1-α-hydroxyvitamin D2 (doxercalciferol), are calcitriol precursors, as they can be activated to 1,25-dihydroxyvitamin D3 or D2, respectively, in the liver, through their hydroxylation at carbon 25. On the other hand, paricalcitol and maxacalcitol are true calcitriol analogs, structurally different compounds that maintain calcitriol selective actions in the control of SHPT with less adverse effects on calcium and phosphate metabolism as reviewed in (43). There are important clinical considerations for interventions using high doses of calcitriol, calcitriol precursors or its analogs: (1) They are incapable of correcting underlying vitamin D deficiency, and (2) their induction of CYP24A1 expression may further reduce not only systemic levels of 25(OH)D for local calcitriol production, but also intracellular calcitriol for VDR activation (see **Figure 1**).

A previously unrecognized synergy between 25(OH)D and calcitriol for VDR activation could be exploited to safely improve clinical outcomes in CKD without escalating calcitriol (analog) doses (see **Figure 1**). Studies in the CYP27B1 null mouse (44), which lack the capacity to convert 25(OH)D to calcitriol, and using 25(OH)D analogs chemically modified to prevent hydroxylation at carbon 1 (45, 46) have demonstrated that 25(OH)D can activate the VDR directly, and more importantly, it synergizes with calcitriol activation of the VDR. This 25(OH)D/calcitriol synergy, achieved by normalizing serum 25(OH)D levels, was shown sufficient to overcome the parathyroid resistance to low doses of the calcitriol analog paricalcitol caused by VDR reductions and accumulation of uremic toxins, even in hyper-phosphatemic experimental CKD (38). Thus, in tissues like the parathyroid glands and monocyte macrophages expressing 25-hydroxylases, and whose activities are not as tightly regulated as renal CYP27B1 and CYP24A1, appropriate vitamin D supplementation will help promote synergistic 25(OH)D/calcitriol interactions by increasing intracellular 25(OH)D levels with minimal, if any, impact on systemic calcium homeostasis. If the capacity for extrahepatic conversion of circulating cholecalciferol to 25(OH)D is tissue specific, current recommendations to achieve certain 25(OH)D levels to improve outcomes may not be accurate. A recent comprehensive review updates our understanding of the epidemiology of native hypovitaminosis D in CKD, current available therapeutic interventions and the existing challenges to achieve an appropriate correction of vitamin D deficiency/insufficiency, not only to ameliorate the progression of SHPT, but also to confer renal and cardiovascular protection improving outcomes (47). A better understanding of the modulators of the tissue specific expression and activity of 25-hydroxylases in CKD could improve current recommendations to enhance the survival benefits of a normal vitamin D status in these patients.

LESSONS FROM THE DEFECTIVE CALCITRIOL SUPPRESSION OF PTH SYNTHESIS AND PARATHYROID HYPERPLASIA IN CKD

The parathyroid gland is the calcium sensor of the body and one of the best studied targets of vitamin D actions, where it inhibits PTH synthesis and secretion and suppresses hyperplastic parathyroid cell growth, thus attenuating bone loss and the propensity to develop vascular calcification that increases mortality rates in the course of CKD. Indeed, in hemodialysis patients, the COSMOS study, a large European prospective study with a 3 year follow up on the clinical handling of more than 6,000 CKD-5D patients, has provided an optimal range of serum PTH that associates with the lowest relative risk of mortality (48). Furthermore, COSMOS has also corroborated the survival benefits associated with correction of serum PTH to achieve values within the optimal range.

Hypocalcemia, hyperphosphatemia and vitamin D deficiency are the main causes of SHPT (49). The calcitriol/VDR complex suppresses PTH synthesis through a direct negative regulation of the PTH gene promoter (18). Vitamin D deficiency (50) and progressive reductions in serum calcitriol in the course of CKD (51) also impair the response of the parathyroid gland to calcium due to reductions in parathyroid content of the calcium sensing receptor (CaSR), a gene directly stimulated by the calcitriol/VDR complex (52). In addition to the control of the calcium/PTH axis, the calcitriol/VDR complex induces FGF23 synthesis by cells of the osteoblastic/osteocyte lineage, providing an additional mechanism to reduce PTH secretion if there is sufficient parathyroid klotho (53), another gene induced by the calcitriol/VDR complex (18).

In individuals with normal renal function, the correction of vitamin D deficiency through cholecalciferol supplementation is sufficient to prevent the elevations in serum PTH. However, in CKD, the recommendations of the KDIGO guidelines to correct vitamin D deficiency prior to initiating therapy with calcitriol often fails to correct serum PTH. In fact, in CKD stages 3–4, daily cholecalciferol doses of 4,000 IU/day for 1 month followed by 2,000 IU for 2 additional months, which effectively corrected serum 25(OH)D from 14 to 37 ng/ml, did not reduce serum PTH, despite achieving normal serum calcitriol levels (54). Instead, in the same CKD3-4 group, 50% of patients receiving 50,000 IU of ergocalciferol (vitamin D2) every 14 days, reached 25(OH)D levels above 35 ng/ml that suppressed PTH (5), while daily doses of 8,000 IU of cholecalciferol for 12 weeks effectively controlled SHPT without hyper-calcemic or hyper-phosphatemic episodes (55). In renal transplant recipients, effective PTH suppression requires 100,000 IU of ergocalciferol every 14 days (56).

Part of the difficulty in normalizing serum PTH with vitamin D supplementation as CKD progresses results from the impact of prolonged hypocalcemia or vitamin D deficiency on parathyroid cell proliferation to meet the requirements for higher serum PTH to normalize serum calcium. Persistent hyperphosphatemia also stimulates parathyroid hyperplasia (57).

The severity of parathyroid cell growth determines not only the degree of SHPT but also contributes to marked reductions in parathyroid VDR, calcium sensing receptor, FGF receptors, and cell membrane klotho, thus impairing PTH suppression in response to the correction of vitamin D deficiency or to therapeutic interventions with calcitriol or its analogs, oral calcium, or by the progressive elevations in FGF23 that take place in the course of CKD. The pathogenic link between parathyroid hyperplasia and VDR reductions and its reversal by synergistic 25(OH)D/calcitriol interactions is summarized in **Figure 2**.

The release of mature TGFα from its transmembrane precursor by ADAM17, an enzyme essential for parathyroid gland development (38), initiates a powerful autocrine loop for excessive TGFα/EGFR-growth signals because TGFα induces its own gene expression (58) and that of the ADAM17 gene (38). TGFα/EGFR-driven increases of oncogenic LIP is responsible for the suppression of VDR gene expression (59) and may contribute to the transformation of parathyroid growth from diffuse to nodular (60). The importance of this pathway to the degree of SHPT and resistance to vitamin D therapy was corroborated in nephrectomized mice harboring a parathyroid-specific EGFR inactivation (35). Importantly, in

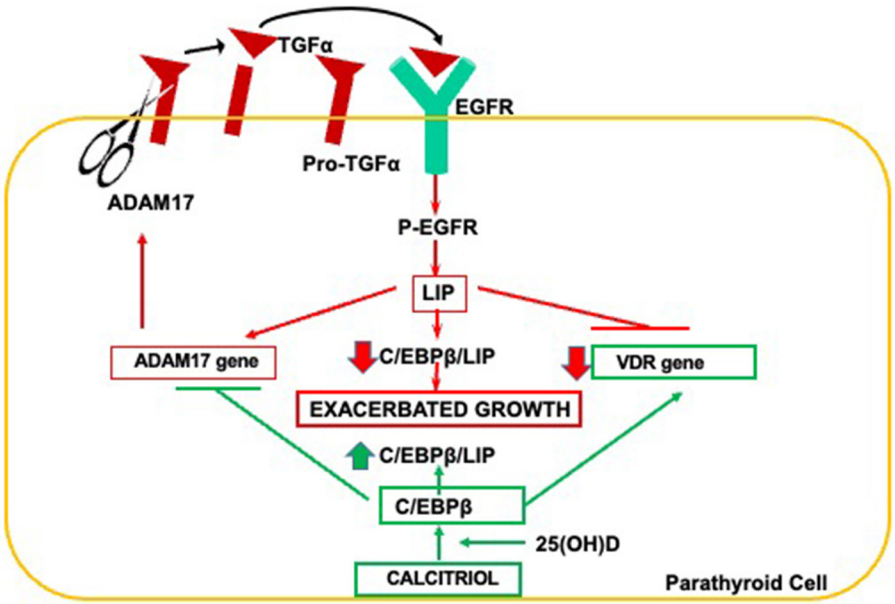


FIGURE 2 | Pathogenesis of parathyroid hyperplasia and resistance to calcitriol actions in CKD. Increases in ADAM17 release of TGFα initiate a vicious ADAM17/TGFα-EGFR cycle responsible for elevations in LIP, the dominant negative isoform of C/EBPβ. Reductions in parathyroid C/EBPβ/LIP ratio induce the ADAM17 gene and suppress VDR gene expression exacerbating parathyroid growth and creating resistance to calcitriol actions. Synergistic interactions between 25(OH)D and calcitriol induce C/EBPβ expression to safely counteract both exacerbated growth and VDR reductions.

a rat model of hyperphosphatemic CKD, when intraperitoneal doses of 25(OH)D that correct vitamin D deficiency but are insufficient to reduce serum PTH, are combined with a paricalcitol dose, also insufficient *per se* to suppress serum PTH or parathyroid cell growth, the 25(OH)D/paricalcitol combination effectively inhibited parathyroid ADAM17 resulting in a 50% PTH reduction (38), despite no changes in serum calcium (38). Mechanistically, improved parathyroid calcitriol synthesis upon vitamin D supplementation, and/or synergistic 25(OH)D/calcitriol interactions that enhance VDR activation partly explain the higher efficacy of the combination to inhibit serum PTH and parathyroid hyperplasia over that of either monotherapy. This synergy may help improve the control of SHPT in advanced CKD patients, whose degree of hypercalcemia or hyperphosphatemia impedes escalating calcitriol or analog dosage. Importantly, a strict control of doses is mandatory, particularly when using oral formulations, as the 25(OH)D/calcitriol (analog) combination will also synergize to activate intestinal VDR increasing calcium and phosphorus absorption. Undoubtedly, the risks of hypercalcemia and hyperphosphatemia will be lower if cholecalciferol, rather than 25(OH)D, is used to normalize vitamin D levels.

DIRECT RENOPROTECTIVE ACTIONS OF THE CALCITRIOL/VDR COMPLEX

Induction of CYP24A1

In the kidney, the induction of CYP24A1 was considered for decades the most critical action of the calcitriol/VDR complex, as it maintains serum calcitriol within normal limits to prevent hypercalcemia and hyperphosphatemia. CYP24A1 degrades excessive circulating levels of calcitriol and also, of 25(OH)D. In agreement with the distinct calcitropic potency of these two vitamin D metabolites, CYP24A1 has a 25-fold higher affinity for calcitriol than for 25(OH)D (18). The severe hypercalcemia and nephrocalcinosis in children and adults with a loss of function mutation of this gene (61, 62), which corroborated the phenotype of the CYP24A1 null mouse (63), strongly supports the clinical significance of calcitriol induction of CYP24A1 in vitamin D responsive tissues.

Induction of Klotho

Progressive reductions in renal content of the longevity gene klotho (64) increase mortality rates in the course of CKD due to accelerated skeletal, immune, renal and cardiovascular aging.

Klotho is expressed mostly in the kidney, the parathyroid gland, and the choroid plexus (65). Interestingly, only renal klotho content appears to be essential for survival, since a kidney specific klotho ablation sufficed to reproduce the accelerated skeletal, immune, renal and cardiovascular aging of the klotho null mice (66). This finding and the identification of a VDRE in the human klotho promoter (67) suggested that defects in vitamin D induction of renal klotho could mediate, in part, the epidemiological association between vitamin D deficiency and higher risk of all-cause mortality in the general population (68), a risk markedly augmented in CKD patients (9). In fact, the lower survival rates in hemodialysis patients carrying a klotho polymorphism that impaired function were improved by

calcitriol (analog) administration (69). However, it is unclear whether the increases in survival involved elevations in the levels of the defective klotho, or direct pro-survival actions of vitamin D interventions unrelated to the induction of renal klotho.

Our understanding of the cross-talk between CKD and loss of klotho pro-survival actions, including protection against CKD progression, has evolved substantially in the last 10 years. Initially, membrane bound renal klotho was considered, exclusively, a high affinity receptor for circulating FGF23 and, therefore, essential for FGF23 phosphaturic actions that attenuate the pro-aging features of hyperphosphatemia: SHPT, systemic inflammation, vascular calcification (70).

Klotho also exists in a soluble form, with FGF23-independent endocrine actions. Soluble klotho is generated by proteolytic cleavage of the transmembrane klotho, is found in blood, urine and cerebrospinal fluid (71, 72), and is associated with identical survival benefits, which also include phosphaturic actions, as well as anti-inflammatory, anti-apoptotic, and anti-oxidant properties (73, 74).

Distinct mechanisms mediate the phosphaturic actions of membrane bound- and soluble klotho (s-klotho). The FGF23/FGFR/membrane bound klotho complex decreases the expression of the sodium-phosphate co-transporter NPT2a at the cell surface of proximal tubular cells reducing phosphate reabsorption (75). Instead, S-klotho cleaves residues in the NPT2a that promote its endocytosis, thus impeding phosphate entrance into renal tubular cells in an FGF23- independent manner (72). Clinically, the importance of the FGF23/klotho and S-klotho actions to attenuate the mortality risks associated with hyperphosphatemia were demonstrated in CKD patients stages 3–4. Progressive reductions of renal klotho impaired the response to high serum FGF23, markedly reducing fractional excretion of phosphate and causing 4-fold higher Kauppila indexes, a measure of abdominal aortic calcification (76). Calcitriol induction of renal klotho will reduce the resistance to FGF23-driven phosphaturia. In addition, calcitriol/VDR induction of renal megalin (22), essential for the endocytosis of the NPT2 co-transporter (77), could synergize with the phosphaturic actions of s-klotho.

In mice with normal kidney function, Klotho induction of autophagy by disruption of the formation of the Beclin1/Bcl2 complex (78), is one of the mechanisms critical for klotho prevention of premature aging and lifespan improvements that are unrelated to the attenuation of hyperphosphatemia. Similar Klotho-mediated increases in authophagic flow could explain why systemic administration of recombinant klotho rescues the renal and cardiovascular damage associated to acute or chronic renal injury (79–81).

Significantly, two mouse models of acute kidney injury (AKI), namely bilateral ischemia reperfusion injury, and unilateral ischemia and unilateral nephrectomy, have demonstrated that klotho/s-klotho induction of autophagic flow also contributes to attenuate AKI progression to CKD. Briefly, it is well-accepted that despite a complete recovery of renal function, AKI later progresses to CKD with decreases in creatinine clearance, hyperphosphatemia, and increases in renal fibrosis. While klotho haplo-insufficient mice progressed to CKD much faster, klotho overexpressing mice were protected. Importantly, administration of recombinant α-klotho also protected mice from AKI-driven CKD mostly through increases in renal cell autophagic flow. However, the anti-oxidant, anti-apoptotic actions of soluble klotho cannot be ruled out (82).

Vitamin D anti-oxidant, pro-autophagic, and anti-apoptotic properties may synergize with klotho actions to protect the kidney from CKD progression (83).

Overall, preventing the reduction of renal and/or circulating klotho is essential to reduce the severity of CKD progression and the risk for cardiovascular mortality. Indeed, serum s-klotho decreases with age, hypertension (84), and systemic inflammation (85), all recognized determinants of renal damage and cardiovascular disease.

The main contribution of the kidney to serum s-klotho levels was strongly supported by an 80% reduction in circulating s-klotho upon specific ablation of renal klotho (66). This finding was critical to consider that serum s-klotho levels could be an accurate biomarker of renal klotho content, CKD progression and of cardiovascular mortality risk in CKD patients. However, the kidney is also the main organ for the clearance of circulating klotho into the urine, through a process of transcytosis through tubular cells (86). Therefore, increases in circulating s-klotho could reflect an impaired transcytosis to the urine by the damaged kidney, which may mask both, actual renal klotho reductions and potential improvements in renal klotho content induced by vitamin D interventions. Therefore, before serum or urinary s- klotho can be used as biomarkers of renal and cardiovascular risk, it will be important to establish the optimal range for serum and urinary s-klotho that are associated with the lowest mortality risks at different CKD stages.

Regarding therapy with calcitriol or its analogs to recover renal klotho in advanced CKD, it is important to consider the severe alterations in the vitamin D/klotho-FGF23 axis in CKD to avoid hyperphosphatemia and excess levels of circulating calcitriol that could compromise klotho pro-survival actions.

CALCITRIOL REDUCTION OF HYPERTENSION-DRIVEN RENAL AND VASCULAR DAMAGE UNRELATED TO THE SUPPRESSION OF THE RENIN GENE

Suppression of Renin

Vitamin D deficiency has been associated with the development of hypertension. Calcitriol/VDR suppression of renin gene expression (87) explains in part the causal association between increases in circulating 25(OH)D levels and reductions in blood pressure and hypertension demonstrated by Mendelian randomization analysis (88). However, randomized controlled trials in individuals with normal renal function have yielded controversial results, partly due to variable vitamin D interventions as well as the inclusion of subjects with normal vitamin D levels or receiving anti-hypertensive medications

(89). In experimental models of diabetic nephropathy, the simultaneous administration of the angiotensin receptor 1 (AT1R) inhibitor Losartan and the calcitriol analog paricalcitol effectively attenuated Losartan-induced compensatory increases in renin, which resulted in much lower serum angiotensin II (90). The efficacy of calcitriol (analog) therapy at decreasing the activation of the RAAS has also demonstrated a beneficial impact on proteinuria, systemic inflammation and the progression of the cardiorenal syndrome (91). However, as will be presented below, the calcitriol (analog)/VDR complex may ameliorate angiotensin II–driven renal damage independently of its downregulation of RAAS activation by suppressing renin gene expression.

Suppression of ADAM17

The pioneer work of Lautrette and collaborators demonstrated that Angiotensin II causes tubular hyperplasia, fibrosis, glomerulosclerosis, proteinuria, and inflammatory infiltration to the renal parenchyma through the enhancement of ADAM17 activity at the surface of renal tubular cells (Summarized in **Figure 3**) (37). Significantly, ADAM17-promoted release of TGFα to drive EGFR activation—the pathway responsible for the onset of parathyroid hyperplasia and resistance to calcitriol/VDR actions—also mediates angiotensin-driven progression of renal injury. In fact, in a mouse model of mild CKD, either ablation of TGFα, inhibition of EGFR activation, or inhibition of ADAM17 markedly reduced the degree of renal injury in response to a prolonged exposure to high levels of angiotensin II, despite persistent hypertension.

Since the activation of renal ADAM17/TGFα signals occurs in human CKD of any etiology (92), calcitriol/VDR suppression of ADAM17 (38) could contribute to ameliorate CKD progression in hypertensive individuals (see **Figure 3**). The combined suppression of renin and ADAM17 gene expression by calcitriol could contribute to the synergy between paricalcitol and the ACE-inhibitor, enalapril. In addition, the increases in renal ADAM17 in CKD also contribute to the renal damage caused by excessive inflammation.

ADAM17, also called TACE for Tumor Necrosis Factor Converting Enzyme, is the enzyme responsible for the release of TNFα to the circulation. Since TNFα induces ADAM17 gene transcription (93), a vicious cycle is initiated to increase renal ADAM17 and TNFα-driven systemic inflammation, further potentiating renal inflammatory damage in an angiotensin II-independent manner. Consequently, this process is no longer responsive to anti-RAS therapy. Calcitriol/VDR suppression of renin, ADAM17 and, also of TNFα gene expression, explain the synergy between the calcitriol analog paricalcitol and the angiotensin II converting enzyme inhibitor enalapril at reducing inflammatory macrophage infiltration in the renal parenchyma in rat CKD (94).

Induction of ACE2

Calcitriol induction of Angiotensin converting enzyme II (ACE2) expression can effectively counteract angiotensin II-driven hypertensive, inflammatory and pro-fibrotic signals, as depicted in **Figure 3**. ACE2 catalyzes the conversion of both angiotensin I and angiotensin II into angiotensin 1-9 and angiotensin 1-7, respectively, thereby reducing circulating levels and the deleterious effects of an excess of angiotensin II that follows RAS hyperactivation (95). In addition to reducing circulating angiotensin II, angiotensin 1-7 binding to its receptor MAS activates signaling pathways that promote anti-fibrotic, anti-oxidant, and vasodilatory signals, thus favoring multiorgan protection and normotension (96, 97).

Calcitriol induction of ACE2 exerts neuroprotective effects in the hypertensive brain by attenuating ROS production and shifting microglia polarization from M1 to M2 (98), and also prevents LPS-induced acute lung injury by attenuating the accelerated neutrophil infiltration and severe inflammation of the lung that follows ACE2 reductions (99). However, in CKD patients with no history of cardiovascular damage, levels of circulating, soluble ACE2 correlated with the classical cardiovascular risk factors (older age, diabetes, male gender) (100). Since ADAM17 cleaves ACE2, the increases in ADAM17 expression and activity that occurs in kidney disease of all etiologies could partly account for the increases in circulating ACE2 and a higher risk for cardiovascular events (100). Conversely, the cardiovascular protection by calcitriol and its analogs could result from suppression of ADAM17 expression and induction of ACE2. In fact, in non-obese diabetic mice, the calcitriol analog paricalcitol, alone or in combination with aliskiren, effectively counteracted the rise in circulating, soluble ACE2 levels associated with diabetes. Reduced ADAM17 expression, oxidative stress and proteinuria (101) were also observed, emphasizing the reno-protective effects of ACE2 induction.

In summary, the actions of the vitamin D endocrine system at mitigating RAS hyperactivation extend far beyond the initial discovery of calcitriol's ability to suppress renin gene expression. Calcitriol exerts reno-protective effects to attenuate CKD progression induced by excessive angiotensin II by (1) counteracting ADAM17/TGFα signals, (2) inducing ACE2/Angiotensin 1-7/MAS receptor activity at the cell surface by preventing ACE2 shedding in the setting of increased ADAM17 activity in uremia and, (3) by preventing excessive renal inflammation through the simultaneous suppression of the vicious TNFα/ADAM17 feed-forward loop. Undoubtedly, in the course of CKD, elevations in circulating ACE2 activity provide a novel biomarker of the failure of vitamin D/calcitriol interventions to effectively suppress the deleterious effects of ADAM17 and RAS hyperactivation.

Despite the recommendation that circulating levels of 25(OH)D above 23 ng/ml may effectively attenuate CKD progression (10), it is imperative to recognize that levels above 35 ng/ml may be necessary to suppress PTH with cholecalciferol supplementation in more advanced CKD, as angiotensin II-driven increases in ADAM17/TGFα signaling may cause renal resistance to calcitriol actions through VDR reductions, as demonstrated in hyperplastic parathyroid glands. Thus, optimal vitamin D intervention may require the synergistic 25(OH)D/calcitriol (analog) interactions through normalization of vitamin D levels and low doses of calcitriol (analogs).

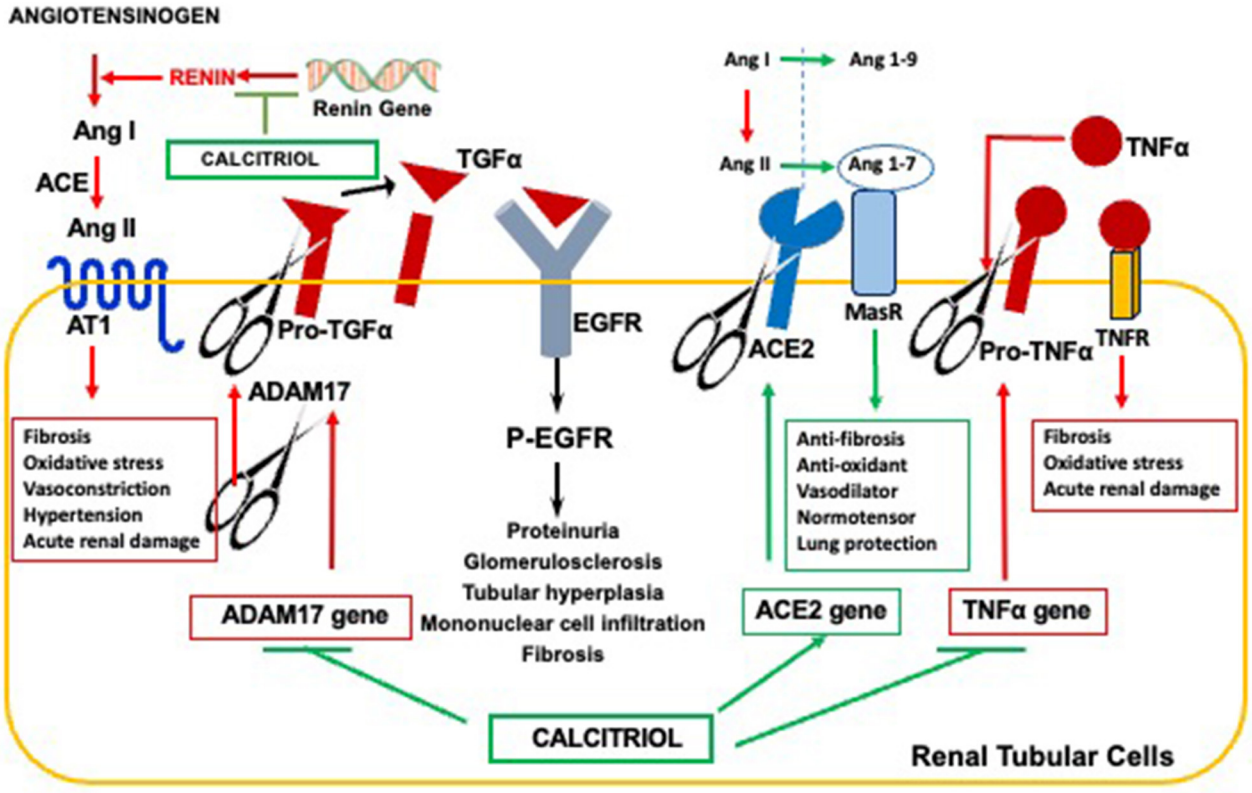


FIGURE 3 | Anti-hypertensive properties of the calcitriol-VDR complex reducing CKD Progression. Calcitriol ameliorates hypertension-driven renal damage by suppression of renin gene expression, responsible for elevations in circulating Angiotensin II; Inhibition of ADAM17 expression to effectively counteract Angiotensin II-mediated increases in TGFα/EGFR signals responsible for CKD progression; Induction of ACE2 for Angiotensin 1-7 synthesis and Angiotenisn 1-7/MAS receptor anti-hypertensive, anti-fibrotic and anti-inflammatory signals, and Inhibition of TNFα expression, whose induction of the ADAM17 gene further augments systemic inflammation and renal injury.

CALCITRIOL ATTENUATION OF IMMUNE CELL FUNCTION AUGMENTING SYSTEMIC INFLAMMATION AND OXIDATIVE STRESS-MEDIATED MULTI-ORGAN DAMAGE

Calcitriol is a well-recognized immunomodulator. A normal vitamin D status is essential for adequate function of the immune system to ensure protection from bacterial and viral infections, minimize progression of autoimmune disorders, and avoid excessive inflammation (102). The benefits of normal vitamin D on innate and acquired immunity involve multiple mechanisms including decreasing the antigenicity of antigen presenting cells, downregulating the production of pro-fibrotic and pro-inflammatory Th1 cytokines, and enhancing the production of anti-inflammatory Th2 cytokines. Calcitriol/VDR acts on T cells to shift their polarization toward a Th2/regulatory phenotype and stimulates T regulatory lymphocyte development (1, 102). Calcitriol inhibition of Skp2 expression is a key mechanism for the maintenance of T regs (103) and may be critical at avoiding the blockage on autophagic flow driven by the cell invasion by Middle East respiratory syndrome coronavirus (MERS-CoV) (104). Calcitriol immune regulatory properties confer protection not only from inflammation-driven multiple organ damage but also from inflammation-driven reductions of renal klotho that accelerate CKD progression.

ADAM17-mediated release of TNFα to the circulation is a main determinant of the degree of systemic inflammation and renal and cardiovascular damage. In fact, a polymorphism of ADAM17 that results in a mild increase in TNFα release is associated with a higher degree of cardiovascular mortality in individuals with normal renal function (105). Furthermore, specific deletion of ADAM17 in myeloid cells markedly reduces mortality rates in a rat model of LPS-induced sepsis (106).

In CKD patients, circulating mononuclear cells express higher levels of ADAM17 and higher serum levels of TNFα, which can be reduced by combined vitamin D and calcitriol (analog) administration (107). These circulating mononuclear cells from hemodialysis patients also have a 40% reduced capacity for 25(OH)D uptake, which mimics impaired 25(OH)D uptake in renal proximal tubular cells with advancing CKD. Interestingly, even when mononuclear cells do not express megalin, the defective uptake was corrected with calcitriol

administration (108), further supporting the use of combined 25(OH)D and calcitriol (analogs) as discussed with hyperplastic parathyroid cells.

In the vasculature, calcitriol suppression of the ADAM17/TNFα loop should decrease TNFα-induction of vascular neutral sphyngomyelinase 2 that drives the release of pro-calcifying exosomes propagating—calcium deposition (109). Moreover, calcitriol induction of miR145 is critical for vascular health, as this is the prevalent miR in vascular smooth muscle cells and the master regulator of their contractile phenotype (110). Reductions in miR145 are associated with vascular diseases and calcification (111). Importantly, decreases in miR145 increase ADAM17 gene expression (112), thus initiating the ADAM17-TNFα loop for vascular injury. It is unclear at present whether monocyte expression of ADAM17, TNFα and neutral sphingomyelinase 2 reflect their vascular levels. Therefore, the assessment of the benefits of vitamin D/calcitriol interventions on vascular health in the course of CKD is limited to less sensitive markers of changes in vascular function, such as pulse wave velocity or Kauppila index. Finally, as indicated earlier, the induction of ADAM17 by increased angiotensin II provides a causal link between hypertension and inflammation. Importantly, as described below, a novel causal link between inflammation driving hypertension has been identified recently.

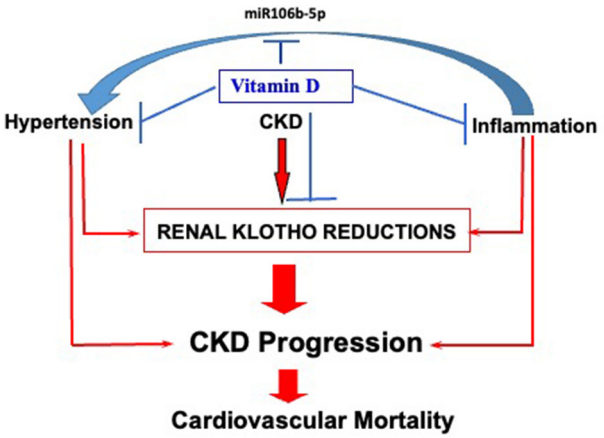


FIGURE 4 | Integration of non-classical vitamin D actions conferring renoprotection. Vitamin D protects against CKD progression through direct inhibition of systemic inflammation, hypertension and renal klotho reductions; indirect attenuation of the reductions in renal klotho content through the control of its most potent downregulators, systemic inflammation and hypertension; inhibition of the release of miR106b-5p by pro-inflammatory monocyte-macrophages, a novel link between inflammation with renin-dependent hypertension.

INFLAMMATION-INDUCED RENIN-DRIVEN HYPERTENSION

Hypertension and inflammation are interrelated processes. In mice, while the absence of monocyte lineage prevents angiotensin II-driven hypertension (113), the overactivation of the renin-angiotensin system results in renal and vascular accumulation of proinflammatory macrophages resulting in increased oxidative stress and its associated tissue damage. In the vasculature, hypertension-induced macrophage infiltration drives nitric oxide scavenging causing reductions in renal blood flow (114), which in turn induce renin secretion by juxtaglomerular cells (114). However, until recently, there was no evidence for immune cell-induced hypertension or its modulation by vitamin D. The pioneer work by Oh and collaborators has demonstrated that increases in ER stress in response to vitamin D deficiency are sufficient to cause renin dependent hypertension through the secretion of micro-RNA 106b-5p that enables a direct communication between innate immune cells and juxtaglomerular cells (115). Importantly, the miR106b-5p link between inflammatory immune cells and renin secretion by juxtaglomerular cells can be prevented by the calcitriol/VDR complex.

In summary, as depicted in **Figure 4**, the integrity of the vitamin D endocrine system protects from CKD progression through direct inhibition of renal klotho reductions, systemic inflammation, and hypertension. Maintenance of renal klotho ensures the reduction of the pro-aging actions of phosphate retention, as well as the longevity properties of klotho-mediated induction of renal autophagic flow. However, circulating soluble klotho is not an accurate marker of renal klotho reductions.

Vitamin D-mediated suppression of hypertension has a dual impact on CKD progression by reducing angiotensin II-driven renal damage and attenuating the reductions in renal klotho. The former is achieved through the maintenance of an adequate balance between pro-hypertensive (Renin and ADAM17) and anti-hypertensive signals (ACE2). Increases in circulating ACE2 levels provide an accurate measurement of increased ADAM17 activity and the cardiovascular risk associated to reductions in ACE2 expression at the cell membrane in CKD, which may be corrected with calcitriol (analog) therapy. Finally, calcitriol reduction of miR106b-5p release to the circulation by pro-inflammatory monocyte-macrophages provides the first causal link between macrophage ER stress activation and the induction of renin-dependent hypertension.

At this time, the optimal supplementation strategy to ensure the desired outcome is not known. This is due to a tremendous variability among individuals in their capacity non only for local calcitriol production, but also for the local bioactivation of vitamin D to 25(OH)D in tissues bearing 25-hydroxylases. To overcome these limitations, it is mandatory to examine whether miR106b, circulating ACE2, angiotensin II, or angiotensin 1-7 levels can better reflect the benefits of vitamin D/calcitriol (analog) interventions and serve as biomarkers to optimize the reno-protective effects of vitamin D therapy in CKD compared to circulating 25(OH)D levels.

AUTHOR CONTRIBUTIONS

AD, KB, and CB-M wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by NIH RO1HL09481806 and VA Merit Award 1BX003648-01 and P30DK020579.

REFERENCES

1. Adams JS, Hewison M. Update in vitamin D. *J Clin Endocrinol Metab.* (2010) 95:471–8. doi: 10.1210/jc.2009-1773
2. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, Kiefte-de-Jong JC, et al. Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. *BMJ.* (2014) 348:g1903. doi: 10.1136/bmj.g1903
3. Ortiz A, Covic A, Fliser D, Fouque D, Goldsmith D, Kanbay M, et al. Epidemiology, contributors to, and clinical trials of mortality risk in chronic kidney failure. *Lancet.* (2014) 383:1831–43. doi: 10.1016/S0140-6736(14)60384-6
4. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant.* (2003) 18:1731–40. doi: 10.1093/ndt/gfg414
5. Al-Badr W, Martin KJ. Vitamin D and kidney disease. *Clin J Am Soc Nephrol.* (2008) 3:1555–60. doi: 10.2215/CJN.01150308
6. Ellam TJ, Chico TJ. Phosphate: the new cholesterol? The role of the phosphate axis in non-uremic vascular disease. *Atherosclerosis.* (2012) 220:310–8. doi: 10.1016/j.atherosclerosis.2011.09.002
7. Hu MC, Kuro-o M, Moe OW. The emerging role of Klotho in clinical nephrology. *Nephrol Dial Transplant.* (2012) 27:2650–7. doi: 10.1093/ndt/gfs160
8. LaClair RE, Hellman RN, Karp SL, Kraus M, Ofner S, Li Q, et al. Prevalence of calcidiol deficiency in CKD: a cross-sectional study across latitudes in the United States. *Am J Kidney Dis.* (2005) 45:1026–33. doi: 10.1053/j.ajkd.2005.02.029
9. Melamed ML, Astor B, Michos ED, Hostetter TH, Powe NR, Muntner P. 25-hydroxyvitamin D levels, race, and the progression of kidney disease. *J Am Soc Nephrol.* (2009) 20:2631–9. doi: 10.1681/ASN.2009030283
10. Nakano C, Hamano T, Fujii N, Matsui I, Tomida K, Mikami S, et al. Combined use of vitamin D status and FGF23 for risk stratification of renal outcome. *Clin J Am Soc Nephrol.* (2012) 7:810–9. doi: 10.2215/CJN.08680811
11. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol.* (2005) 289:F8–28. doi: 10.1152/ajprenal.00336.2004
12. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab.* (1988) 67:373–8. doi: 10.1210/jcem-67-2-373
13. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *Proc Natl Acad Sci USA.* (2004) 101:7711–5. doi: 10.1073/pnas.0402490101
14. Brown AJ, Ritter CS, Knutson JC, Strugnell SA. The vitamin D prodrugs 1alpha(OH)D2, 1alpha(OH)D3 and BCI-210 suppress PTH secretion by bovine parathyroid cells. *Nephrol Dial Transplant.* (2006) 21:644–50. doi: 10.1093/ndt/gfi186
15. Bergada L, Pallares J, Maria Vittoria A, Cardus A, Santacana M, Valls J, et al. Role of local bioactivation of vitamin D by CYP27A1 and CYP2R1 in the control of cell growth in normal endometrium and endometrial carcinoma. *Lab Invest.* (2014) 94:608–22. doi: 10.1038/labinvest.2014.57
16. Heaney RP, Armas LA, Shary JR, Bell NH, Binkley N, Hollis BW. 25-Hydroxylation of vitamin D3: relation to circulating vitamin D3 under various input conditions. *Am J Clin Nutr.* (2008) 87:1738–42. doi: 10.1093/ajcn/87.6.1738
17. Dusso AS, Tokumoto M. Defective renal maintenance of the vitamin D endocrine system impairs vitamin D renoprotection: a downward spiral in kidney disease. *Kidney Int.* (2011) 79:715–29. doi: 10.1038/ki.2010.543
18. Haussler MR, Whitfield GK, Kaneko I, Haussler CA, Hsieh D, Hsieh JC, et al. Molecular mechanisms of vitamin D action. *Calcif Tissue Int.* (2013) 92:77–98. doi: 10.1007/s00223-012-9619-0
19. Jacob AI, Sallman A, Santiz Z, Hollis BW. Defective photoproduction of cholecalciferol in normal and uremic humans. *J Nutr.* (1984) 114:1313–9. doi: 10.1093/jn/114.7.1313
20. Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell.* (1999) 96:507–15. doi: 10.1016/S0092-8674(00)80655-8
21. Takemoto F, Shinki T, Yokoyama K, Inokami T, Hara S, Yamada A, et al. Gene expression of vitamin D hydroxylase and megalin in the remnant kidney of nephrectomized rats. *Kidney Int.* (2003) 64:414–20. doi: 10.1046/j.1523-1755.2003.00114.x
22. Liu W, Yu WR, Carling T, Juhlin C, Rastad J, Ridefelt P, et al. Regulation of gp330/megalin expression by vitamins A and D. *Eur J Clin Invest.* (1998) 28:100–7. doi: 10.1046/j.1365-2362.1998.00253.x
23. Halloran BP, Schaefer P, Lifschitz M, Levens M, Goldsmith RS. Plasma vitamin D metabolite concentrations in chronic renal failure: effect of oral administration of 25-hydroxyvitamin D3. *J Clin Endocrinol Metab.* (1984) 59:1063–9. doi: 10.1210/jcem-59-6-1063
24. Dusso A, Lopez-Hilker S, Rapp N, Slatopolsky E. Extra-renal production of calcitriol in chronic renal failure. *Kidney Int.* (1988) 34:368–75. doi: 10.1038/ki.1988.190
25. Krajisnik T, Bjorklund P, Marsell R, Ljunggren O, Akerstrom G, Jonsson KB, et al. Fibroblast growth factor-23 regulates parathyroid hormone and 1alpha-hydroxylase expression in cultured bovine parathyroid cells. *J Endocrinol.* (2007) 195:125–31. doi: 10.1677/JOE-07-0267
26. Dai B, David V, Alshayeb HM, Showkat A, Gyamlani G, Horst RL, et al. Assessment of 24,25(OH)2D levels does not support FGF23-mediated catabolism of vitamin D metabolites. *Kidney Int.* (2012) 82:1061–70. doi: 10.1038/ki.2012.222
27. Bosworth CR, Levin G, Robinson-Cohen C, Hoofnagle AN, Ruzinski J, Young B, et al. The serum 24,25-dihydroxyvitamin D concentration, a marker of vitamin D catabolism, is reduced in chronic kidney disease. *Kidney Int.* (2012) 82:693–700. doi: 10.1038/ki.2012.193
28. Saramaki A, Diermeier S, Kellner R, Laitinen H, Vaisanen S, Carlberg C. Cyclical chromatin looping and transcription factor association on the regulatory regions of the p21 (CDKN1A) gene in response to 1alpha,25-dihydroxyvitamin D3. *J Biol Chem.* (2009) 284:8073–82. doi: 10.1074/jbc.M808090200
29. Kim S, Yamazaki M, Zella LA, Shevde NK, Pike JW. Activation of receptor activator of NF-kappaB ligand gene expression by 1,25-dihydroxyvitamin D3 is mediated through multiple long-range enhancers. *Mol Cell Biol.* (2006) 26:6469–86. doi: 10.1128/MCB.00353-06
30. Centeno PP, Herberger A, Mun HC, Tu C, Nemeth EF, Chang W, et al. Phosphate acts directly on the calcium-sensing receptor to stimulate parathyroid hormone secretion. *Nat Commun.* (2019) 10:4693. doi: 10.1038/s41467-019-12399-9
31. Giangreco AA, Nonn L. The sum of many small changes: microRNAs are specifically and potentially globally altered by vitamin D3 metabolites. *J Steroid Biochem Mol Biol.* (2013) 136:86–93. doi: 10.1016/j.jsbmb.2013.01.001
32. Liu X, Cheng Y, Yang J, Qin S, Chen X, Tang X, et al. Flank sequences of miR-145/143 and their aberrant expression in vascular disease: mechanism and therapeutic application. *J Am Heart Assoc.* (2013) 2:e000407. doi: 10.1161/JAHA.113.000407
33. Taibi F, Metzinger-Le Meuth V, M'Baya-Moutoula E, Djelouat M, Louvet L, Bugnicourt JM, et al. Possible involvement of microRNAs in vascular

damage in experimental chronic kidney disease. *Biochim Biophys Acta.* (2014) 1842:88–98. doi: 10.1016/j.bbadis.2013.10.005
34. Rangrez AY, M'Baya-Moutoula E, Metzinger-Le Meuth V, Henaut L, Djelouat MS, Benchitrit J, et al. Inorganic phosphate accelerates the migration of vascular smooth muscle cells: evidence for the involvement of miR-223. *PLoS ONE.* (2012) 7:e47807. doi: 10.1371/journal.pone.0047807
35. Arcidiacono MV, Yang J, Fernandez E, Dusso A. Parathyroid-specific epidermal growth factor-receptor inactivation prevents uremia-induced parathyroid hyperplasia in mice. *Nephrol Dial Transplant.* (2015) 30:434–40. doi: 10.1093/ndt/gfu318
36. Gooz M. ADAM-17: the enzyme that does it all. *Crit Rev Biochem Mol Biol.* (2010) 45:146–69. doi: 10.3109/10409231003628015
37. Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, et al. Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. *Nat Med.* (2005) 11:867–74. doi: 10.1038/nm1275
38. Arcidiacono MV, Yang J, Fernandez E, Dusso A. The induction of C/EBPbeta contributes to vitamin D inhibition of ADAM17 expression and parathyroid hyperplasia in kidney disease. *Nephrol Dial Transplant.* (2015) 30:423–33. doi: 10.1093/ndt/gfu311
39. Haussler MR, Whitfield GK, Kaneko I, Forster R, Saini R, Hsieh JC, et al. The role of vitamin D in the FGF23, klotho, and phosphate bone-kidney endocrine axis. *Rev Endocr Metab Disord.* (2012) 13:57–69. doi: 10.1007/s11154-011-9199-8
40. Dusso AS. Vitamin D receptor: mechanisms for vitamin D resistance in renal failure. *Kidney Int Suppl.* (2003) S6–9. doi: 10.1046/j.1523-1755.63.s85.3.x
41. Dusso AS, Negrea L, Gunawardhana S, Lopez-Hilker S, Finch J, Mori T, et al. On the mechanisms for the selective action of vitamin D analogs. *Endocrinology.* (1991) 128:1687–92. doi: 10.1210/endo-128-4-1687
42. Teng M, Wolf M, Ofsthun MN, Lazarus JM, Hernan MA, Camargo CA, et al. Activated injectable vitamin d and hemodialysis survival: a historical cohort study. *J Am Soc Nephrol.* (2005) 16:1115–25. doi: 10.1681/ASN.2004070573
43. Cozzolino M, Ketteler M, Zehnder D. The vitamin D system: a crosstalk between the heart and kidney. *Eur J Heart Fail.* (2010) 12:1031–41. doi: 10.1093/eurjhf/hfq112
44. Hoenderop JG, Chon H, Gkika D, Bluyssen HA, Holstege FC, St-Arnaud R, et al. Regulation of gene expression by dietary Ca2+ in kidneys of 25-hydroxyvitamin D3-1 alpha-hydroxylase knockout mice. *Kidney Int.* (2004) 65:531–9. doi: 10.1111/j.1523-1755.2004.00402.x
45. Lou YR, Molnár F, Peräkylä M, Qiao S, Kalueff AV, St-Arnaud R, et al. 25-Hydroxyvitamin D(3) is an agonistic vitamin D receptor ligand. *J Steroid Biochem Mol Biol.* (2010) 118:162–70. doi: 10.1016/j.jsbmb.2009.11.011
46. Munetsuna E, Nakabayashi S, Kawanami R, Yasuda K, Ohta M, Arai MA, et al. Mechanism of the anti-proliferative action of 25-hydroxy-19-nor-vitamin D(3) in human prostate cells. *J Mol Endocrinol.* (2011) 47:209–18. doi: 10.1530/JME-11-0008
47. Alfieri C, Ruzhytska O, Vettoretti S, Caldiroli L, Cozzolino M, Messa P. Native Hypovitaminosis D in CKD patients: from experimental evidence to clinical practice. *Nutrients.* (2019) 11:1918. doi: 10.3390/nu11081918
48. Fernandez-Martin JL, Martinez-Camblor P, Dionisi MP, Floege J, Ketteler M, London G, et al. Improvement of mineral and bone metabolism markers is associated with better survival in haemodialysis patients: the COSMOS study. *Nephrol Dial Transplant.* (2015) 30:1542–51. doi: 10.1093/ndt/gfv099
49. Slatopolsky E, Brown A, Dusso A. Role of phosphorus in the pathogenesis of secondary hyperparathyroidism. *Am J Kidney Dis.* (2001) 37:S54–7. doi: 10.1053/ajkd.2001.20740
50. Brown AJ, Zhong M, Finch J, Ritter C, McCracken R, Morrissey J, et al. Rat calcium-sensing receptor is regulated by vitamin D but not by calcium. *Am J Physiol.* (1996) 270:F454–60. doi: 10.1152/ajprenal.1996.270.3.F454
51. Kifor O, Moore FD Jr, Wang P, Goldstein M, Vassilev P, Kifor I, et al. Reduced immunostaining for the extracellular Ca2+-sensing receptor in primary and uremic secondary hyperparathyroidism. *J Clin Endocrinol Metab.* (1996) 81:1598–606. doi: 10.1210/jcem.81.4.8636374
52. Canaff L, Hendy GN. Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J Biol Chem.* (2002) 277:30337–50. doi: 10.1074/jbc.M201804200
53. Canalejo R, Canalejo A, Martinez-Moreno JM, Rodriguez-Ortiz ME, Estepa JC, Mendoza FJ, et al. FGF23 fails to inhibit uremic parathyroid glands. *J Am Soc Nephrol.* 21:1125–35. doi: 10.1681/ASN.2009040427
54. Moe SM, Saifullah A, LaClair RE, Usman SA, Yu Z. A randomized trial of cholecalciferol versus doxercalciferol for lowering parathyroid hormone in chronic kidney disease. *Clin J Am Soc Nephrol.* (2010) 5:299–306. doi: 10.2215/CJN.07131009
55. Westerberg PA, Sterner G, Ljunggren O, Isaksson E, Elvarson F, Dezfoolian H, et al. High doses of cholecalciferol alleviate the progression of hyperparathyroidism in patients with CKD Stages 3-4: results of a 12-week double-blind, randomized, controlled study. *Nephrol Dial Transplant.* (2018) 33:466–71. doi: 10.1093/ndt/gfx059
56. Courbebaisse M, Thervet E, Souberbielle JC, Zuber J, Eladari D, Martinez F, et al. Effects of vitamin D supplementation on the calcium-phosphate balance in renal transplant patients. *Kidney Int.* (2009) 75:646–51. doi: 10.1038/ki.2008.549
57. Slatopolsky E, Finch J, Denda M, Ritter C, Zhong M, Dusso A, et al. Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion *in vitro*. *J Clin Invest.* (1996) 97:2534–40. doi: 10.1172/JCI118701
58. Arcidiacono MV, Cozzolino M, Spiegel N, Tokumoto M, Yang J, Lu Y, et al. Activator Protein 2{alpha} Mediates Parathyroid TGF-{alpha} Self-Induction in Secondary Hyperparathyroidism. *J Am Soc Nephrol.* (2008) 19:1919–28. doi: 10.1681/ASN.2007111216
59. Arcidiacono MV, Sato T, Alvarez-Hernandez D, Yang J, Tokumoto M, Gonzalez-Suarez I, et al. EGFR activation increases parathyroid hyperplasia and calcitriol resistance in kidney disease. *J Am Soc Nephrol.* (2008) 19:310–20. doi: 10.1681/ASN.2007040406
60. Zahnow CA, Cardiff RD, Laucirica R, Medina D, Rosen JM. A role for CCAAT/enhancer binding protein beta-liver-enriched inhibitory protein in mammary epithelial cell proliferation. *Cancer Res.* (2001) 61:261–9.
61. Jacobs TP, Kaufman M, Jones G, Kumar R, Schlingmann KP, Shapses S, et al. A lifetime of hypercalcemia and hypercalciuria, finally explained. *J Clin Endocrinol Metab.* (2014) 99:708–12. doi: 10.1210/jc.2013-3802
62. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med.* (2011) 365:410–21. doi: 10.1056/NEJMoa1103864
63. St-Arnaud R, Arabian A, Travers R, Barletta F, Raval-Pandya M, Chapin K, et al. Deficient mineralization of intramembranous bone in vitamin D-24- hydroxylase-ablated mice is due to elevated 1,25-dihydroxyvitamin D and not to the absence of 24,25-dihydroxyvitamin D. *Endocrinology.* (2000) 141:2658–66. doi: 10.1210/endo.141.7.7579
64. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature.* (1997) 390:45–51. doi: 10.1038/36285
65. Li SA, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K. Immunohistochemical localization of Klotho protein in brain, kidney, and reproductive organs of mice. *Cell Struct Funct.* (2004) 29:91–9. doi: 10.1247/csf.29.91
66. Lindberg K, Amin R, Moe OW, Hu MC, Erben RG, Ostman Wernerson A, et al. The kidney is the principal organ mediating klotho effects. *J Am Soc Nephrol.* (2014) 25:2169–75. doi: 10.1681/ASN.2013111209
67. Forster RE, Jurutka PW, Hsieh JC, Haussler CA, Lowmiller CL, Kaneko I, et al. Vitamin D receptor controls expression of the anti-aging klotho gene in mouse and human renal cells. *Biochem Biophys Res Commun.* (2011) 414:557–62. doi: 10.1016/j.bbrc.2011.09.117
68. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med.* (2008) 168:1629–37. doi: 10.1001/archinte.168.15.1629
69. Friedman DJ, Afkarian M, Tamez H, Bhan I, Isakova T, Wolf M, et al. Klotho variants and chronic hemodialysis mortality. *J Bone Miner Res.* (2009) 24:1847–55. doi: 10.1359/jbmr.090516
70. Kurosu H, Kuro-o M. The Klotho gene family and the endocrine fibroblast growth factors. *Curr Opin Nephrol Hypertens.* (2008) 17:368–72. doi: 10.1097/MNH.0b013e3282ffd994
71. Imura A, Iwano A, Tohyama O, Tsuji Y, Nozaki K, Hashimoto N, et al. Secreted Klotho protein in sera and CSF: implication for post-translational

cleavage in release of Klotho protein from cell membrane. *FEBS Lett.* (2004) 565:143–7. doi: 10.1016/j.febslet.2004.03.090
72. Hu MC, Shi M, Zhang J, Pastor J, Nakatani T, Lanske B, et al. Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. *FASEB J.* (2010) 24:3438–50. doi: 10.1096/fj.10-154765
73. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone Klotho. *Science.* (2005) 309:1829–33. doi: 10.1126/science.1112766
74. Hu MC, Kuro-o M, Moe OW. Renal and extrarenal actions of Klotho. *Semin Nephrol.* (2013) 33:118–29. doi: 10.1016/j.semnephrol.2012.12.013
75. Razzaque MS, Lanske B. The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis. *J Endocrinol.* (2007) 194:1–10. doi: 10.1677/JOE-07-0095
76. Craver L, Dusso A, Martinez-Alonso M, Sarro F, Valdivielso JM, Fernandez E. A low fractional excretion of Phosphate/Fgf23 ratio is associated with severe abdominal Aortic calcification in stage 3 and 4 kidney disease patients. *BMC Nephrol.* (2013) 14:221. doi: 10.1186/1471-2369-14-221
77. Bachmann S, Schlichting U, Geist B, Mutig K, Petsch T, Bacic D, et al. Kidney-specific inactivation of the megalin gene impairs trafficking of renal inorganic sodium phosphate cotransporter (NaPi-IIa). *J Am Soc Nephrol.* (2004) 15:892–900. doi: 10.1097/01.ASN.0000120389.09938.21
78. Fernandez AF, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, et al. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. *Nature.* (2018) 558:136–40. doi: 10.1038/s41586-018-0162-7
79. Hu MC, Shi M, Cho HJ, Adams-Huet B, Paek J, Hill K, et al. Klotho and phosphate are modulators of pathologic uremic cardiac remodeling. *J Am Soc Nephrol.* (2015) 26:1290–302. doi: 10.1681/ASN.2014050465
80. Hu MC, Shi M, Zhang J, Quinones H, Griffith C, Kuro-o M, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* (2011) 22:124–36. doi: 10.1681/ASN.2009121311
81. Hu MC, Shi M, Zhang J, Quinones H, Kuro-o M, Moe OW. Klotho deficiency is an early biomarker of renal ischemia-reperfusion injury and its replacement is protective. *Kidney Int.* (2010) 78:1240–51. doi: 10.1038/ki.2010.328
82. Shi M, Flores B, Gillings N, Bian A, Cho HJ, Yan S, et al. alphaKlotho mitigates progression of AKI to CKD through activation of autophagy. *J Am Soc Nephrol.* (2016) 27:2331–45. doi: 10.1681/ASN.2015060613
83. Cannell JJ, Grant WB, Holick MF. Vitamin D and inflammation. *Dermatoendocrinology.* (2014) 6:e983401. doi: 10.4161/19381980.2014.983401
84. Mitani H, Ishizaka N, Aizawa T, Ohno M, Usui S, Suzuki T, et al. *In vivo* klotho gene transfer ameliorates angiotensin II-induced renal damage. *Hypertension.* (2002) 39:838–43. doi: 10.1161/01.HYP.0000013734.33441.EA
85. Izquierdo MC, Perez-Gomez MV, Sanchez-Nino MD, Sanz AB, Ruiz-Andres O, Poveda J, et al. Klotho, phosphate and inflammation/ageing in chronic kidney disease. *Nephrol Dial Transplant.* (2012) 4:iv6–10. doi: 10.1093/ndt/gfs426
86. Hu MC, Shi M, Zhang J, Addo T, Cho HJ, Barker SL, et al. Renal production, uptake, and handling of circulating alphaKlotho. *J Am Soc Nephrol.* (2016) 27:79–90. doi: 10.1681/ASN.2014101030
87. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* (2002) 110:229–38. doi: 10.1172/JCI0215219
88. Vimaleswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. *Lancet Diabetes Endocrinol.* (2014) 2:719–29. doi: 10.1016/S2213-8587(14)70113-5
89. Rejnmark L, Bislev LS, Cashman KD, Eiriksdottir G, Gaksch M, Grubler M, et al. Non-skeletal health effects of vitamin D supplementation: a systematic review on findings from meta-analyses summarizing trial data. *PLoS ONE.* (2017) 12:e0180512. doi: 10.1371/journal.pone.0180512
90. Zhang Z, Sun L, Wang Y, Ning G, Minto AW, Kong J, et al. Renoprotective role of the vitamin D receptor in diabetic nephropathy. *Kidney Int.* (2008) 73:163–71. doi: 10.1038/sj.ki.5002572
91. Ronco C, Cozzolino M. Mineral metabolism abnormalities and vitamin D receptor activation in cardiorenal syndromes. *Heart Fail Rev.* (2012) 17:211–20. doi: 10.1007/s10741-011-9232-8
92. Melenhorst WB, Visser L, Timmer A, van den Heuvel MC, Stegeman CA, van Goor H. ADAM17 upregulation in human renal disease: a role in modulating TGF-{alpha} availability? *Am J Physiol Renal Physiol.* (2009) 297:F781–90. doi: 10.1152/ajprenal.90610.2008
93. Charbonneau M, Harper K, Grondin F, Pelmus M, McDonald PP, Dubois CM. Hypoxia-inducible factor mediates hypoxic and tumor necrosis factor alpha-induced increases in tumor necrosis factor-alpha converting enzyme/ADAM17 expression by synovial cells. *J Biol Chem.* (2007) 282:33714–24. doi: 10.1074/jbc.M704041200
94. Mizobuchi M, Morrissey J, Finch JL, Martin DR, Liapis H, Akizawa T, et al. Combination therapy with an Angiotensin-converting enzyme inhibitor and a vitamin d analog suppresses the progression of renal insufficiency in uremic rats. *J Am Soc Nephrol.* (2007) 18:1796–806. doi: 10.1681/ASN.2006091028
95. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci USA.* (2003) 100:8258–63. doi: 10.1073/pnas.1432869100
96. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem.* (2002) 277:14838–43. doi: 10.1074/jbc.M200581200
97. Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD, et al. The emerging role of ACE2 in physiology and disease. *J Pathol.* (2007) 212:1–11. doi: 10.1002/path.2162
98. Cui C, Xu P, Li G, Qiao Y, Han W, Geng C, et al. Vitamin D receptor activation regulates microglia polarization and oxidative stress in spontaneously hypertensive rats and angiotensin II-exposed microglial cells: role of renin-angiotensin system. *Redox Biol.* (2019) 26:101295. doi: 10.1016/j.redox.2019.101295
99. Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, et al. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg(9) bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. *Am J Physiol Lung Cell Mol Physiol.* (2018) 314:L17–31. doi: 10.1152/ajplung.00498.2016
100. Anguiano L, Riera M, Pascual J, Valdivielso JM, Barrios C, Betriu A, et al. Circulating angiotensin-converting enzyme 2 activity in patients with chronic kidney disease without previous history of cardiovascular disease. *Nephrol Dial Transplant.* (2015) 30:1176–85. doi: 10.1093/ndt/gfv025
101. Riera M, Anguiano L, Clotet S, Roca-Ho H, Rebull M, Pascual J, et al. Paricalcitol modulates ACE2 shedding and renal ADAM17 in NOD mice beyond proteinuria. *Am J Physiol Renal Physiol.* (2016) 310:F534–46. doi: 10.1152/ajprenal.00082.2015
102. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev.* (2008) 29:726–76. doi: 10.1210/er.2008-0004
103. Wang D, Qin H, Du W, Shen YW, Lee WH, Riggs AD, et al. Inhibition of S-phase kinase-associated protein 2 (Skp2) reprograms and converts diabetogenic T cells to Foxp3+ regulatory T cells. *Proc Natl Acad Sci USA.* (2012) 109:9493–8. doi: 10.1073/pnas.1207293109
104. Gassen NC, Niemeyer D, Muth D, Corman VM, Martinelli S, Gassen A, et al. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. *Nat Commun.* (2019) 10:5770. doi: 10.1038/s41467-019-13659-4
105. Morange PE, Tregouet DA, Godefroy T, Saut N, Bickel C, Rupprecht HJ, et al. Polymorphisms of the tumor necrosis factor-alpha (TNF) and the TNF-alpha converting enzyme (TACE/ADAM17) genes in relation to cardiovascular mortality: the AtheroGene study. *J Mol Med.* (2008) 86:1153–61. doi: 10.1007/s00109-008-0375-6
106. Horiuchi K, Kimura T, Miyamoto T, Takaishi H, Okada Y, Toyama Y, et al. Cutting edge: TNF-alpha-converting enzyme (TACE/ADAM17) inactivation in mouse myeloid cells prevents lethality from endotoxin shock. *J Immunol.* (2007) 179:2686–9. doi: 10.4049/jimmunol.179.5.2686
107. Dusso A, Arcidiacono MV, Yang J, Tokumoto M. Vitamin D inhibition of TACE and prevention of renal osteodystrophy and cardiovascular mortality. *J Steroid Biochem Mol Biol.* (2010) 121:193–8. doi: 10.1016/j.jsbmb.2010.03.064

108. Gallieni M, Kamimura S, Ahmed A, Bravo E, Delmez J, Slatopolsky E, et al. Kinetics of monocyte 1 alpha-hydroxylase in renal failure. *Am J Physiol.* (1995) 268:F746–53. doi: 10.1152/ajprenal.1995.268.4.F746
109. Kapustin AN, Chatrou ML, Drozdov I, Zheng Y, Davidson SM, Soong D, et al. Vascular smooth muscle cell calcification is mediated by regulated exosome secretion. *Circ Res.* (2015) 116:1312–23. doi: 10.1161/CIRCRESAHA.116.305012
110. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature.* (2009) 460:705–10. doi: 10.1038/nature08195
111. Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ Res.* (2009) 105:158–66. doi: 10.1161/CIRCRESAHA.109.197517
112. Doberstein K, Steinmeyer N, Hartmetz AK, Eberhardt W, Mittelbronn M, Harter PN, et al. MicroRNA-145 targets the metalloprotease ADAM17 and is suppressed in renal cell carcinoma patients. *Neoplasia.* (2013) 15:218–30. doi: 10.1593/neo.121222
113. De Ciuceis C, Amiri F, Brassard P, Endemann DH, Touyz RM, Schiffrin EL. Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II-infused macrophage colony-stimulating factor-deficient mice: evidence for a role in inflammation in angiotensin-induced vascular injury. *Arterioscler Thromb Vasc Biol.* (2005) 25:2106–13. doi: 10.1161/01.ATV.0000181743.28028.57
114. Norlander AE, Madhur MS, Harrison DG. Correction: the immunology of hypertension. *J Exp Med.* (2018) 215:719. doi: 10.1084/jem.2017177301022018c
115. Oh J, Matkovich SJ, Riek AE, Bindom SM, Shao JS, Head RD, et al. Macrophage secretion of miR-106b-5p causes renin-dependent hypertension. *Nat Commun.* (2020) 11:4798. doi: 10.1038/s41467-020-18538-x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

MINI REVIEW
published: 20 December 2021
doi: 10.3389/fmed.2021.777861

Sodium-Glucose Cotransporter 2 Inhibitors Mechanisms of Action: A Review

*Jorge I. Fonseca-Correa and Ricardo Correa-Rotter**

Department of Nephrology and Mineral Metabolism, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

Sodium-Glucose Cotransporter 2 inhibitors (SGLT2i), or gliflozins, are a group of antidiabetic drugs that have shown improvement in renal and cardiovascular outcomes in patients with kidney disease, with and without diabetes. In this review, we will describe the different proposed mechanisms of action of SGLT2i. Gliflozins inhibit renal glucose reabsorption by blocking the SGLT2 cotransporters in the proximal tubules and causing glucosuria. This reduces glycemia and lowers HbA_{1c} by ∼1.0%. The accompanying sodium excretion reverts the tubuloglomerular feedback and reduces intraglomerular pressure, which is central to the nephroprotective effects of SGLT2i. The caloric loss reduces weight, increases insulin sensitivity, lipid metabolism, and likely reduces lipotoxicity. Metabolism shifts toward gluconeogenesis and ketogenesis, thought to be protective for the heart and kidneys. Additionally, there is evidence of a reduction in tubular cell glucotoxicity through reduced mitochondrial dysfunction and inflammation. SGLT2i likely reduce kidney hypoxia by reducing tubular energy and oxygen demand. SGLT2i improve blood pressure through a negative sodium and water balance and possibly by inhibiting the sympathetic nervous system. These changes contribute to the improvement of cardiovascular function and are thought to be central in the cardiovascular benefits of SGLT2i. Gliflozins also reduce hepcidin levels, improving erythropoiesis and anemia. Finally, other possible mechanisms include a reduction in inflammatory markers, fibrosis, podocyte injury, and other related mechanisms. SGLT2i have shown significant and highly consistent benefits in renal and cardiovascular protection. The complexity and interconnectedness of the primary and secondary mechanisms of action make them a most interesting and exciting pharmacologic group.

Keywords: SGLT2 inhibitors, gliflozins, kidney disease, heart failure, diabetes

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Daw-Yang Hwang, National Health Research Institutes, Taiwan

****Correspondence:***
Ricardo Correa-Rotter
correarotter@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *15 September 2021*
Accepted: *22 November 2021*
Published: *20 December 2021*

Citation:
Fonseca-Correa JI and Correa-Rotter R (2021) Sodium-Glucose Cotransporter 2 Inhibitors Mechanisms of Action: A Review. Front. Med. 8:777861. doi: 10.3389/fmed.2021.777861

INTRODUCTION

Sodium Glucose Cotransporter 2 inhibitors (SGLT2i), also known as gliflozins, are an exciting and highly interesting group of "relatively new drugs" that have shown consistent positive results in renal and cardiovascular protection. They inhibit the action of the Sodium Glucose Cotransporter 2 (SGLT2) in the kidney and cause glucosuria. Initially, they were thought of and developed as glucose lowering therapies, yet large clinical trials in a very short time have demonstrated clinical benefits that far exceeded what was expected. In this short review we describe the physiologic effects of SGLT2 inhibitors and discuss the clinical benefits demonstrated to date.

PHYSIOLOGY OF SGLT2 COTRANSPORTERS AND RENAL GLUCOSE HANDLING

SGLT2 cotransporters are part of a large family of symporters responsible for facilitated transport of different solutes, aided by a positive sodium gradient (1, 2). There are two main sodium-glucose cotransporters in the body: SGLT1 and SGLT2. SGLT2 cotransporters are almost exclusively found in renal tissue, whereas SGLT1 are mostly found in the small intestine, heart, and skeletal muscle, aside from the kidney (**Table 1**) (3–5). In the kidneys, SGLT2 and SGLT1 handle sodium and glucose reabsorption in the proximal tubules of the nephron. Their physiological function is to reabsorb 100% of filtered glucose, avoiding energy loss through glucosuria. SGLT2 cotransporters are found in the brush border of the renal tubular cells in the first segments of the proximal tubules (S1 and S2). They have a high transport capacity but low affinity for glucose and are responsible for the reabsorption of 90 to 97% of filtered glucose. The remaining 3–10% of filtered glucose is absorbed by the high-affinity, low-capacity, SGLT1 cotransporter, present in the S3 segment of the proximal tubule. Glucose exits these tubular cells back into the circulation through the GLUT2 (for cells with SGLT2) and GLUT1 (for cells with SGLT1) transporters in the basoletaral membrane. This unidirectional transport of glucose and sodium is coupled to, and maintained by, the Na-K-ATPase pump in the basolateral membrane.

The coupled work between the high-capacity/low-affinity SGLT2 and low-capacity/high-affinity SGLT1 cotransporters handles the full load of filtered glucose. This way, in a normal physiological setting, glucose reabsorption by the proximal tubules is adjusted to the variations in serum glucose concentrations. Total glucose reabsorption is directly proportional to the amount of filtered glucose. This reabsorption capacity has a natural limit ($Tmax_G$) that is reached when filtered glucose approximates 350 mg/min/1.73 m^2, equivalent to between 180 and 200 mg/dL of glycemia. Glucosuria develops when hyperglycemia exceeds this $Tmax_G$. In chronic hyperglycemia of diabetes, the kidney shifts the $Tmax_G$ to higher glucose levels, around 240 mg/dL (6). The proximal tubules increase the number of SGLT2 cotransporters to make up for the increase in luminal glucose flow (7). This increase in SGLT2 cotransporters comes at a cost of energy expenditure through the basolateral Na-K-ATPase, and is thought to be central to the pathophysiology of diabetic kidney disease (8).

SGLT2 COTRANSPORTER INHIBITORS MECHANISMS OF ACTION

The first SGLT2 inhibitor was named phlorizin, a naturally occurring phenolic glycoside derived from the root bark of the apple tree (9). It was first isolated in the 19th century and was originally thought to have antipyretic properties. Further analysis found that phlorizin causes glucosuria and it was thought to cause a diabetes-like state when administered to dogs, due to the presence of glucosuria, polyuria and weight loss.

With the characterization of renal glucose reabsorption in the proximal tubule in the 1960's, the cloning of the SGLT2 cotransporter in the 1990's (10), and further understanding of renal handling of glucose and the pharmacological effects of phlorizin, inhibition of renal glucose reabsorption was studied as a target for diabetes control. Preclinical studies in the 1980's showed that phlorizin improved insulin sensitivity in diabetic rat models without affecting insulin action in control rats (11).

Phlorizin has no oral bioavailability, so it can only be administered intravenously. The first orally available SGLT2 inhibitor, T-1095, was developed in the 1990's. It showed some improvement in HbA1c, reduction of microalbuminuria, and weight loss in rats (12, 13). Unfortunately, T-1095 was not selective to SGLT2 and its action on intestinal SGLT1 caused significant gastrointestinal adverse effects and intolerance. Following T-1095, at least seven different orally available SGLT2 inhibitors have been developed (2), three of which have been approved for use by the FDA: dapagliflozin, empagliflozin, and canagliflozin. The three of them are highly selective for SGLT2 inhibition over SGLT1.

Several clinical trials with empagliflozin (14–17), canagliflozin (18, 19), and dapagliflozin (20–22) in the past few years have demonstrated impressive benefits from SGLT2 inhibition in high-cardiovascular-risk patients. They have shown significant reduction of cardiovascular and all-cause mortality, hospitalizations for heart failure, adverse cardiovascular events, and progression of albuminuria, when added to standard therapy in diabetic and non-diabetic kidney disease. Particularly interesting is the fact that the use of SGLT2 has proven beneficial for kidney disease and heart failure despite the absence of diabetes as a central pathology. Understanding the direct and indirect physiological mechanisms and effects of SGLT2 inhibition is crucial to clarify why they offer a diversity of clinical benefits.

Direct Physiological Effects of SGLT2 Inhibition

Glucosuria: Improvement in Glucose Control

Inhibition of SGLT2 cotransporter causes glucosuria. By inhibiting the SGLT2 cotransporter, gliflozins avoid glucose reabsorption in the S1 and S2 segments of the proximal tubule. This causes a reduction in $Tmax_G$ to around 40–80 mg/dL (6) and a reduction in the renal threshold for glucosuria. To avoid significant energy loss through glucosuria, SLGT1 cotransporters compensate by increasing reabsorption to ∼40% (23). A preclinical study in rats demonstrated this by showing that double SGTL1/SGLT2-knockout mice have significantly higher glucosuria than single SGLT2-knockout mice (24). Furthermore, glucose control from SGLT2 inhibition is not significantly associated with a higher risk of hypoglycemia (25).

Glucose control improvement is reflected by a reduction in HbA_{1c} of ∼0.5–1% (14, 18, 19, 25, 26). This leads to an increased sensitivity to insulin and enhanced beta-cell function (27–30), and is of relevance in the role of SGLT2 inhibition in diabetes control. All the main clinical trials on SGLT2 inhibitors (14–16, 18–22) have demonstrated a consistent benefit in glucose

TABLE 1 | Main differences between SGLT2 and SGLT1 cotransporters.

	SGLT2	SGLT1
Gene	*SCL5A2* (16p11.2)	*SLC5A1* (22p13.1)
Tissue	Kidney* (To a lower extent: brain, liver, thyroid, pancreas, skeletal muscle).	Small intestine*, heart, skeletal muscle, trachea, kidney, brain, testicles and prostate.
Selectivity	Glucose	Glucose, galactose
Capacity/affinity for glucose transport	High/Low	Low/High
Na:Glucose transport stoichiometry	1:1	2:1
Physiological function	Reabsorption of renal filtered glucose	Intestinal absorption of glucose and galactose; reabsorption of renal filtered glucose
Percentage of renal glucose reabsorption	90–97%	3–10% (up to 40% with SGLT2 inhibition)

*Main location.
Adapted from references (3–5).

control. The benefit is evident even when added to standard therapy (31).

Natriuresis: Improvement in Blood Pressure and Reversal of Tubuloglomerular Feedback Stimulation

Together with glucosuria, SGLT2 inhibition causes natriuresis that is associated with a negative salt and water balance (32). This reduction in plasma volume is evidenced by a drop in blood pressure of 3–6 mmHg in systolic and 1–1.5 mmHg in diastolic (18, 19) blood pressures.

Increased natriuresis and sodium delivery to the distal nephron is central for renal protection, as it normalizes the tubuloglomerular feedback mechanism. Chronic hyperglycemia of diabetes induces a state of increased reabsorption in the proximal tubule by increasing the SGLT2 cotransporter expression [and the $Tmax_G$ (6)]. This increased glucose and sodium reabsorption reduces the delivery of sodium to the juxtaglomerular apparatus, stimulating the tubuloglomerular feedback, which in turn causes dilation of the afferent arteriole trying to "normalize" distal sodium delivery. Dilation of the afferent arteriole increases intraglomerular pressure and causes hyperfiltration, characteristic of diabetic kidney disease. SGLT2 inhibition reverses this feedback loop by increasing sodium delivery to the juxtaglomerular apparatus, inhibiting the tubuloglomerular feedback and causing constriction of the afferent arteriole (6, 33). The result is a reduction in intraglomerular pressure and improvement of hyperfiltration, that is reflected as an initial drop of glomerular filtration rate (GFR). This drop in GFR is reversible when SGLT2 inhibition is discontinued and is a response to hemodynamic changes. Although this initial GFR drop associated to SGLT2 inhibition may seem significant, its magnitude is limited in most clinical instances to 2–4 ml/min. Studies with long term follow up show that it is not continuous and is significantly less than the eGFR decline observed in the placebo groups.

Secondary Effects of SGLT2 Inhibition

Improvement in Albuminuria

Clinical trials on diabetic and non-diabetic patients with chronic kidney disease (CKD) (22, 34) have demonstrated that SGLT2 inhibitors reduce albuminuria significantly. This effect is independent and additive to the effect of RAAS blockade (15). The improvement in albuminuria is multifactorial, related to the vasoconstriction of the afferent arteriole, the subsequent reduction in intraglomerular pressure and hyperfiltration, as well as the improvement in systemic blood pressure.

Some studies have also suggested that podocytes benefit from SGLT2 inhibition, as they have SGLT2 cotransporters, and the use of dapagliflozin or empagliflozin reduces podocyte dysfunction and effacement (35, 36) through normalization in insulin sensitivity and improvement in glucotoxicity. This would lead to an improvement in albuminuria (37).

Weight Loss and Lipid Metabolism Shift

The use of SGLT2 inhibitors induces weight loss of between 2 and 4 kg after 6–12 months of treatment (25, 26, 29, 38–41). Initial weight loss is related to volume contraction and subsequently secondary to caloric wasting through glucosuria. ADA guidelines actually recommend SGLT2 inhibitors as initial antidiabetic therapy when weight loss is desired as part of the treatment (42).

SGLT2 inhibition and the subsequent glucosuria induces a state of relative glucose "deprivation," shifting energetic substrate use to lipids. This reduces cellular lipotoxicity and improves oxidative stress. This also favors an increase in ketone production, which appear to be a better energetic substrate for renal and myocardial cells.

Improvement in Proximal Tubular Work and Oxygen Consumption

As described previously, hyperglycemia of diabetes induces a state of glucose and sodium hyperreabsorption in the proximal tubule, activates tubuloglomerular feedback and causes hyperfiltration. This positive feedback loop increases cellular work due to increased Na-K-ATPase activity and induces proximal tubular hypertrophy. Aside from this, increased intracellular glucose in proximal tubular cells is diverted to non-glycolytic pathways increasing advanced glycation end-products, affecting mitochondrial activity, and increasing oxidative stress. Inhibition of the tubuloglomerular feedback, hyperfiltration and increased glucose reabsorption reduces energy expenditure and

oxygen consumption in proximal cells (8). Reducing serum and intracellular glucose levels, reduces cellular glucotoxicity.

Improved Oxygen Delivery and Anemia

The improvement in proximal tubular cell work and reduction in energy expenditure described above, reduces oxygen demand and increases cortical oxygen tension (8, 43). Subsequent delivery of glucose to the latter part of the proximal tubule is reabsorbed by the SGLT1 cotransporters and increases energy expenditure and oxygen consumption in the renal outer medulla (44–46). The decrease in oxygen availability stimulates hypoxia-inducible factors HIF1 and HIF2 (47) and enhance the release of erythropoietin (48). This, together with a mild volume contraction, increases the hemoglobin and favors oxygen delivery to different tissues. Clinical trials have shown improvement in hemoglobin levels in patients treated with SGLT2i (49). Dapagliflozin appears to suppress hepcidin and other iron-metabolism related proteins, helping improve erythropoiesis (50).

Other Possible Effects

Gliflozins appear to reduce inflammatory markers such as IL-6, TNF, IFNγ, NF-κβ, TLR-4, and TGF-β (51–54). They also appear to improve mitochondrial function (55) reduce mesangial expansion and the number of myofibroblast in myocardial tissue (56). Empagliflozin appears to reduce IL-β inflammatory pathway in proximal tubular cells (57). These effects would reduce inflammation, fibrosis, and oxidative stress in myocardial and renal tissue. Nevertheless, all these changes appear to be secondary to the metabolic and hemodynamic effects of SGLT2 inhibition.

Adverse Effects Associated to SGLT2 Inhibition

The use of SGLT2 inhibitors is associated with adverse events that are rare and generally mild, yet should be considered. Volume contraction and osmotic diuresis are direct effects of their mechanism of action and may infrequently present of a magnitude of significance when the drug is initiated in geriatric patients and in those on diuretics. In some clinical trials, a particularly relevant yet infrequent, adverse effect described was euglycemic diabetic ketoacidosis, yet this was not present in CREDENCE and DAPA-CKD. Finally, genital mycotic infections are up to four times more frequent in patients using SGLT2 inhibitors. They are generally mild and easily treatable. Patients should be counseled to monitor signs and symptoms and to maintain adequate genital hygiene. Other adverse events such as bone fractures and amputations appeared to be associated to SGLT2 inhibition in the CANVAS trial, but they have not been replicated in other studies.

CLINICAL BENEFITS OF SGLT2 INHIBITION

Several clinical trials have demonstrated important cardiovascular and renal benefits of SGLT2 inhibition. The EMPA-REG OUTCOME (14) trial was the first study to demonstrate this in a large scale. Published in 2015, this double-blind, multicenter, clinical trial was aimed at demonstrating cardiovascular safety of empagliflozin when added to standard therapy in high cardiovascular risk diabetic patients with an estimated glomerular filtration rate (eGFR) $\geq$30 ml/min/1.73 m^2. After a 3 year follow up, empagliflozin not only proved to be safe, but improved major cardiovascular events (cardiovascular death, myocardial infarction, or stroke) by 14%, reduced all-cause mortality by 32%, cardiovascular death by 37%, and hospitalization due to heart failure by 35%. A subsequent analysis of secondary outcomes (15) confirmed that empagliflozin reduced incident or worsening diabetic nephropathy by 39%. Baseline eGFR declined in subjects on both the placebo and empagliflozin arms, yet this decline in eGFR stabilized after a few weeks in the empagliflozin group and was reverted after empagliflozin was discontinued, showing that the change in eGFR is not related to kidney injury but to hemodynamic changes induced by the drug itself. These results were notable considering that renal outcomes were not the primary outcome of the trial and 80% of subjects were already under RAAS blockade as standard therapy for diabetic kidney disease.

Similarly, the CANVAS (18) trial, in 2017, demonstrated that canagliflozin added to standard therapy in high cardiovascular risk patients reduced mayor cardiovascular events by 14%, cardiovascular death by 13%, myocardial infarction and stroke by 14 and 10% respectively, and hospitalization for heart failure by 33%. Subjects on canagliflozin had a 40% lower risk of adverse renal outcomes (renal function decline, dialysis initiation or death from a renal cause), 27% lower risk of worsening albuminuria and a 1.7 higher likelihood of improvement in albuminuria.

In 2019, the DECLARE-TIMI 58 (20) trial, which included over 17,000 patients followed for 4.2 years, showed that dapagliflozin reduced the risk of hospitalization for heart failure by 27% and of adverse renal outcomes by 27%. The large population of diabetic patients included in this trial represents the widest range of renal function in any of the cardiovascular outcome studies.

An interesting meta-analysis (58) of these trials show that SGLT2 inhibitors significantly reduced the risk of kidney failure by 29%, end-stage kidney disease by 32% and acute kidney injury by 25%. These benefits were consistent across the different subgroups of GFR and albuminuria. Altogether, these studies demonstrate cardiovascular and renal benefit from SGLT2 inhibition in patients with diabetes and high cardiovascular risk, with and without established diabetic nephropathy. Another meta-analysis (59) of the EMPA-REG OUTCOME, CANVAS and DECLARE-TIMI 58 trials stratified the subjects (n = 34,322) according to eGFR and demonstrated that SGLT2 inhibitors had a better effect in reducing adverse renal outcomes (worsening renal function, ESKD or death from renal cause) when eGFR is between 30 and 60 ml/min/1.73 m^2.

The CREDENCE (19) trial, published in 2019, was the first designed to focus on a composite renal outcome that included end stage kidney disease (ESKD) (dialysis requirement, kidney transplantation or eGFR of <15 ml/min/1.73 m^2), doubling of

serum creatinine or renal or cardiovascular death. Similar to CANVAS and EMPA-REG OUTCOME, subjects were diabetic, yet patients in this study had eGFR between 30 and 90 ml/min; 60% of which had to have an eGFR between 30 and 60 ml/min, and all subjects had urinary albumin-creatinine ratio (UACR) between 300 and 5,000 mg/g and optimal renin angiotensin system (RAAS) inhibition. Canagliflozin was associated with a significantly lower risk of adverse renal and cardiovascular outcomes, and the results were so evident and encouraging that, after interim analysis, the trial was stopped prematurely after 2.6 years of follow-up, displaying a reduction of 34% of the primary composite outcome in the canagliflozin group. A secondary analysis (60) of patients in the CREDENCE trial demonstrated that subjects receiving canagliflozin had a lower risk of renal and cardiovascular outcomes even when starting treatment with eGFR between 30 and <45 ml/min/1.73 m^2.

In 2020, the DAPA-CKD (22) study was published. It included over 4,000 CKD patients, comprised 68% by diabetics and 32% by patients with CKD not related to diabetes, with an eGFR of 25–75 ml/min/1.73 m^2 and UACR of 200–5,000 mg/g, treated with dapagliflozin or placebo. The study, as CREDENCE, was stopped prematurely due to the clear benefit offered by dapagliflozin in both diabetic and non-diabetic patients with CKD. The primary renal outcome (sustained reduction of at least 50% of eGFR, ESKD or renal or cardiovascular death) was significantly lower (HR: 0.61; 95% CI: 0.51–0.72; $p < 0.001$) in dapagliflozin treated patients. Benefits were also independent of the baseline presence of cardiovascular disease (61). A prespecified subanalysis (62) of subjects with eGFR <30 ml/min/1.73 m^2 showed that dapagliflozin is safe and effective even in this lower eGFR levels. Similarly, the subgroup of patients with IgA nephropathy (63) treated with dapagliflozin had a lower risk of kidney disease progression, with similar safety profiles when compared to placebo.

There have been important studies to explore of the effects of SGLT2 inhibition on patients with high cardiovascular risk, with or without kidney disease and independently of the presence of diabetes. The DAPA-HF (21) and the EMPEROR-reduced (16) trials demonstrated that the use of either dapagliflozin or empagliflozin reduces cardiovascular death and worsening of heart failure in patients with heart failure and reduced ejection fraction, regardless of the presence or not of diabetes. More recently the EMPEROR-preserved (17) trial also demonstrated similar benefits from empagliflozin in patients with heart failure and preserved ejection fraction.

CONCLUSIONS

The positive results discussed above have been clearly striking and consistent, demonstrating a significant improvement in cardiovascular and renal outcomes by the different SGLT2 inhibitors, when added to optimized standard therapy that includes maximal RAAS inhibition.

The cascade of events induced by the inhibition of SGLT2 cotransporters has proven beneficial to reduce cardiovascular and renal outcomes, and death in patients with and without diabetes. The exact mechanisms of cardiovascular as well as renal benefits are probably related to multiple interplaying factors, but are not completely understood (64). These include a reduction in glycemia with subsequent improvement in insulin resistance, weight loss and reduced visceral fat. Correction of glycemia reduces direct glucotoxicity and has shown improvement in cellular function in proximal renal tubular cells as well as other tissues.

Although SGLT2 inhibition favors an improvement in HbA_{1c}, the extent of this improvement is not enough to explain the significant clinical benefits observed in cardio-renal health. Similarly, hyperglycemia would not be a central pathophysiological issue in patients without diabetes. A possible energetic benefit is the shift to lipid metabolism, with a subsequent reduction in lipotoxicity, as well as an increase in ketone production. Central to the cardiovascular benefit is the diuretic and natriuretic effect of SGLT2 inhibition. The described improvement in volume status, sodium balance and blood pressure seem to be of relevance to both the cardiovascular as well as renal benefits. Reduction in albuminuria, inflammation and oxidative stress have also been implicated. In addition, for the renal component, the intrarenal hemodynamic mechanisms described seem to be key for the long-term improvement in the eGFR slope decline, in diabetic as well as non-diabetic patients.

AUTHOR CONTRIBUTIONS

JF-C and RC-R contributed equally to conception and initial discussions regarding the main subject matter, manuscript focus, and general outline. JF-C performed the initial literature review and drafted the first version of the manuscript and was in charge of writing the final manuscript. RC-R oversaw the structure and logical sequencing of the manuscript, perfected the drafting and ensured that the information was up to date, and added sections to the initial draft. Both authors read and approved the final submitted version.

REFERENCES

1. Santer R, Calado J. Familial renal glucosuria and SGLT2: from a Mendelian trait to a therapeutic target. *Clin J Am Soc Nephrol.* (2010) 5:133–41. doi: 10.2215/CJN.04010609
2. Gallo LA, Wright EM, Vallon V. Probing SGLT2 as a therapeutic target for diabetes: basic physiology and consequences. *Diabetes Vasc Dis Res.* (2015) 12:78–89. doi: 10.1177/1479164114561992
3. Chen J, Williams S, Ho S, Loraine H, Hagan D, Whaley JM, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. *Diabetes Ther.* (2010) 1:57–92. doi: 10.1007/s13300-010-0006-4
4. Wright EM, Hirayama BA, Loo DF. Active sugar transport in health and disease. *J Intern Med.* (2007) 261:32–43. doi: 10.1111/j.1365-2796.2006.01746.x
5. Bakris GL, Fonseca VA, Sharma K, Wright EM. Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications. *Kidney Int.* (2009) 75:1272–7. doi: 10.1038/ki.2009.87
6. Defronzo RA, Hompesch M, Kasichayanula S, Liu X, Hong Y, Pfister M, et al. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. *Diabetes Care.* (2013) 36:3169–76. doi: 10.2337/dc13-0387

7. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes.* (2005) 54:3427–34. doi: 10.2337/diabetes.54.12.3427
8. Vallon V, Thomson SC. The tubular hypothesis of nephron filtration and diabetic kidney disease. *Nat Rev Nephrol.* (2020) 16:317–36. doi: 10.1038/s41581-020-0256-y
9. Ehrenkranz JRL, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. *Diabetes Metab Res Rev.* (2005) 21:31–8. doi: 10.1002/dmrr.532
10. Ghezzi C, Loo DDF, Wright EM. Physiology of renal glucose handling via SGLT1, SGLT2 and GLUT2 online mendelian inheritance in man. *Diabetologia.* (2018) 1:2087–97. doi: 10.1007/s00125-018-4656-5
11. Rossetti L, Smith D, Shulman GI, Papachristou D, DeFronzo RA. Correction of hyperglycemia with phlorizin normalizes tissues sensitivity to insulin in diabetic rats. *J Clin Invest.* (1987) 79:1510–5. doi: 10.1172/JCI112981
12. Adachi T, Yasuda K, Okamoto Y, Shihara N, Oku A, Ueta K, et al. T-1095, A renal Na+-glucose transporter inhibitor, improves hyperglycemia in streptozotocin-induced diabetic rats. *Metabolism.* (2000) 49:990–5. doi: 10.1053/meta.2000.7729
13. Yasuda K, Okamoto Y, Nunoi K, Adachi T, Shihara N, Tamon A, et al. Normalization of cytoplasmic calcium response in pancreatic β-cells of spontaneously diabetic GK rat by the treatment with T-1095, a specific inhibitor of renal Na+-glucose co-transporters. *Horm Metab Res.* (2002) 34:217–21. doi: 10.1055/s-2002-26714
14. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720
15. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, Von Eynatten M, Mattheus M, et al. Empagliflozin and progression of kidney disease in type 2 diabetes. *N Engl J Med.* (2016) 375:323–34. doi: 10.1056/NEJMoa1515920
16. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med.* (2020) 383:1413–24. doi: 10.1056/NEJMoa2022190
17. Anker SD, Butler J, Filippatos G, Ferreira JP, Bocchi E, Böhm M, et al. Empagliflozin in heart failure with a preserved ejection fraction. *N Engl J Med.* (2021) 2021:1–11. doi: 10.1056/NEJMoa2107038
18. Neal B, Perkovic V, Mahaffey KW, De Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* (2017) 377:644–57. doi: 10.1056/NEJMoa1611925
19. Perkovic V, Jardine MJ, Neal B, Bompoint S, Heerspink HJL, Charytan DM, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med.* (2019) 380:2295–306. doi: 10.1056/NEJMoa1811744
20. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* (2019) 380:347–57. doi: 10.1056/NEJMoa1812389
21. McMurray JJV, Solomon SD, Inzucchi SE, Kober L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med.* (2019) 381:1995–2008. doi: 10.1056/NEJMoa1911303
22. Heerspink HJL, Stefánsson B V, Correa-Rotter R, Chertow GM, Greene T, Hou F-F, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med.* (2020) 383:1436–46. doi: 10.1056/NEJMoa2024816
23. Abdul-Ghani MA, DeFronzo RA, Norton L. Novel hypothesis to explain why SGLT2 inhibitors inhibit only 30-50% of filtered glucose load in humans. *Diabetes.* (2013) 62:3324–8. doi: 10.2337/db13-0604
24. Powell DR, DaCosta CM, Gay J, Ding ZM, Smith M, Greer J, et al. Improved glycemic control in mice lacking Sglt1 and Sglt2. *Am J Physiol Endocrinol Metab.* (2013) 304:117–30. doi: 10.1152/ajpendo.00439.2012
25. Del Prato S, Nauck M, Durán-Garcia S, Maffei L, Rohwedder K, Theuerkauf A, et al. Long-term glycaemic response and tolerability of dapagliflozin versus a sulphonylurea as add-on therapy to metformin in patients with type 2 diabetes: 4-year data. *Diabetes, Obes Metab.* (2015) 17:581–90. doi: 10.1111/dom.12459
26. DeFronzo RA, Norton L, Abdul-Ghani M. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. *Nat Rev Nephrol.* (2017) 13:11–26. doi: 10.1038/nrneph.2016.170
27. Kahn BB, Shulman GI, DeFronzo RA, Cushman SW, Rossetti L. Normalization of blood glucose in diabetic rats with phlorizin treatment reverses insulin-resistant glucose transport in adipose cells without restoring glucose transporter gene expression. *J Clin Invest.* (1991) 87:561–70. doi: 10.1172/JCI115031
28. Ferrannini E, Muscelli E, Frascerra S, Baldi S, Mari A, Heise T, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest.* (2014) 124:499–508. doi: 10.1172/JCI72227
29. Merovci A, Solis-Herrera C, Daniele G, Eldor R, Vanessa Fiorentino T, Tripathy D, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest.* (2014) 124:509–14. doi: 10.1172/JCI70704
30. Merovci A, Mari A, Solis C, DeFronzo RA, Daniele G, Chavez-Velazquez A, et al. Dapagliflozin lowers plasma glucose concentration and improves β-cell function. *J Clin Endocrinol Metab.* (2015) 100:1927–32. doi: 10.1210/jc.2014-3472
31. Rosenstock J, Hansen L, Zee P, Li Y, Cook W, Hirshberg B, et al. Dual add-on therapy in type 2 diabetes poorly controlled with metformin monotherapy: a randomized double-blind trial of saxagliptin plus dapagliflozin addition versus single addition of saxagliptin or dapaglif lozin to metformin. *Diabetes Care.* (2015) 38:376–83. doi: 10.2337/dc14-1142
32. Lambers Heerspink H, de Zeeuw D, Wie L, Leslie B, List J. Original Article : Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. *Diabetes, Obes Metab.* (2013) 15:853–62. doi: 10.1111/dom.12127
33. Škrtić M, Yang GK, Perkins BA, Soleymanlou N, Lytvyn Y, von Eynatten M, et al. Characterisation of glomerular haemodynamic responses to SGLT2 inhibition in patients with type 1 diabetes and renal hyperfiltration. *Diabetologia.* (2014) 57:2599–602. doi: 10.1007/s00125-014-3396-4
34. Cherney D, Lund SS, Perkins BA, Groop PH, Cooper ME, Kaspers S, et al. The effect of sodium glucose cotransporter 2 inhibition with empagliflozin on microalbuminuria and macroalbuminuria in patients with type 2 diabetes. *Diabetologia.* (2016) 59:1860–70. doi: 10.1007/s00125-016-4008-2
35. Cassis P, Locatelli M, Cerullo D, Corna D, Buelli S, Zanchi C, et al. SGLT2 inhibitor dapagliflozin limits podocyte damage in proteinuric nondiabetic nephropathy. *JCI insight.* (2018) 3:98720. doi: 10.1172/jci.insight.98720
36. Korbut AI, Taskaeva IS, Bgatova NP, Muraleva NA, Orlov NB, Dashkin M V., et al. SGLT2 inhibitor empagliflozin and DPP4 inhibitor linagliptin reactivate glomerular autophagy in db/db mice, a model of type 2 diabetes. *Int J Mol Sci.* (2020) 21:2987. doi: 10.3390/ijms21082987
37. DeFronzo RA, Reeves WB, Awad AS. Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. *Nat Rev Nephrol.* (2021) 17:319–34. doi: 10.1038/s41581-021-00393-8
38. Abdul-Ghani MA, Norton L, DeFronzo RA. Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. *Endocr Rev.* (2011) 32:515–31. doi: 10.1210/er.2010-0029
39. Vasilakou D, Karagiannis T, Athanasiadou E, Mainou M, Laikos A, Bekiari E, et al. Sodium–Glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis despoina. *Ann Intern Med.* (2013) 159:262–74. doi: 10.7326/0003-4819-159-4-201308200-00007
40. Scheen AJ. Pharmacodynamics, efficacy and safety of sodium-glucose co-transporter type 2 (SGLT2) inhibitors for the treatment of type 2 diabetes mellitus. *Drugs.* (2015) 75:33–59. doi: 10.1007/s40265-014-0337-y
41. Abdul-Ghani MA, Norton L, DeFronzo RA. Efficacy and safety of SGLT2 inhibitors in the treatment of type 2 diabetes mellitus. *Curr Diab Rep.* (2012) 12:230–8. doi: 10.1007/s11892-012-0275-6
42. Care D, Suppl SS. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes 2021. *Diabetes Care.* (2021) 44:S111–24. doi: 10.2337/dc21-S009
43. Nespoux J, Vallon V. SGLT2 inhibition and kidney protection. *Clin Sci.* (2018) 132:1329–39. doi: 10.1042/CS20171298
44. O'Neill J, Fasching A, Pihl L, Patinha D, Franzén S, Palm F. Acute SGLT inhibition normalizes O2 tension in the renal cortex but causes hypoxia in the renal medulla in anaesthetized control and diabetic rats. *Am J Physiol Ren Physiol.* (2015) 309:F227–34. doi: 10.1152/ajprenal.00689.2014
45. Layton AT, Vallon V, Edwards A. Modeling oxygen consumption in the proximal tubule: effects of NHE and SGLT2 inhibition. *Am J Physiol Ren Physiol.* (2015) 308:F1343–57. doi: 10.1152/ajprenal.00007.2015
46. Layton AT, Vallon V, Edwards A. Predicted consequences of diabetes and SGLT inhibition on transport and oxygen consumption along

a rat nephron. *Am J Physiol Ren Physiol.* (2016) 310:F1269–83. doi: 10.1152/ajprenal.00543.2015
47. Vallon V, Rose M, Gerasimova M, Satriano J, Platt KA, Koepsell H, et al. Knockout of Na-glucose transporter SGLT2 attenuates hyperglycemia and glomerular hyperfiltration but not kidney growth or injury in diabetes mellitus. *Am J Physiol Ren Physiol.* (2013) 304:156–67. doi: 10.1152/ajprenal.00409.2012
48. Sano M, Takei M, Shiraishi Y, Suzuki Y. Increased hematocrit during sodium-glucose cotransporter 2 inhibitor therapy indicates recovery of tubulointerstitial function in diabetic kidneys. *J Clin Med Res.* (2016) 8:844–7. doi: 10.14740/jocmr2760w
49. Stefánsson B V, Heerspink HJL, Wheeler DC, Sjöström CD, Greasley PJ, Sartipy P, et al. Correction of anemia by dapagliflozin in patients with type 2 diabetes. *J Diabetes Complications.* (2020) 34:1–7. doi: 10.1016/j.jdiacomp.2020.107729
50. Ghanim H, Abuaysheh S, Hejna J, Green K, Batra M, Makdissi A, et al. Dapagliflozin suppresses hepcidin and increases erythropoiesis. *J Clin Endocrinol Metab.* (2020) 105:E1056–63. doi: 10.1210/clinem/dgaa057
51. Bonnet F, Scheen AJ. Effects of SGLT2 inhibitors on systemic and tissue low-grade inflammation: the potential contribution to diabetes complications and cardiovascular disease. *Diabetes Metab.* (2018) 44:457–64. doi: 10.1016/j.diabet.2018.09.005
52. Yaribeygi H, Butler AE, Atkin SL, Katsiki N, Sahebkar A. Sodium–glucose cotransporter 2 inhibitors and inflammation in chronic kidney disease: possible molecular pathways. *J Cell Physiol.* (2018) 234:223–30. doi: 10.1002/jcp.26851
53. Panchapakesan U, Pegg K, Gross S, Komala MG, Mudaliar H, Forbes J, et al. Effects of SGLT2 inhibition in human kidney proximal tubular cells-renoprotection in diabetic nephropathy? *PLoS ONE.* (2013) 8:2–9. doi: 10.1371/journal.pone.0054442
54. Lee N, Heo YJ, Choi SE, Jeon JY, Han SJ, Kim DJ, et al. Anti-inflammatory effects of empagliflozin and gemigliptin on LPS-stimulated macrophage via the IKK/NF- κ B, MKK7/JNK, and JAK2/STAT1 signalling pathways. *J Immunol Res.* (2021) 2021:9944880. doi: 10.1155/2021/9944880
55. Takagi S, Li J, Takagaki Y, Kitada M, Nitta K, Takasu T, et al. Ipragliflozin improves mitochondrial abnormalities in renal tubules induced by a high-fat diet. *J Diabetes Investig.* (2018) 9:1025–32. doi: 10.1111/jdi.12802
56. Lee TM, Chang NC, Lin SZ. Dapagliflozin, a selective SGLT2 Inhibitor, attenuated cardiac fibrosis by regulating the macrophage polarization via STAT3 signaling in infarcted rat hearts. *Free Radic Biol Med.* (2017) 104:298–310. doi: 10.1016/j.freeradbiomed.2017.01.035
57. Pirklbauer M, Sallaberger S, Staudinger P, Corazza U, Leierer J, Mayer G, et al. Empagliflozin inhibits il-1β-mediated inflammatory response in human proximal tubular cells. *Int J Mol Sci.* (2021) 22:1–12. doi: 10.3390/ijms22105089
58. Neuen BL, Young T, Heerspink HJL, Neal B, Perkovic V, Billot L, et al. SGLT2 inhibitors for the prevention of kidney failure in patients with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol.* (2019) 7:845–54. doi: 10.1016/S2213-8587(19)30256-6
59. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet.* (2019) 393:31–9. doi: 10.1016/S0140-6736(18)32590-X
60. Jardine MJ, Zhou Z, Mahaffey KW, Oshima M, Agarwal R, Bakris G, et al. Renal, cardiovascular, and safety outcomes of canagliflozin by baseline kidney function: a secondary analysis of the CREDENCE randomized trial. *J Am Soc Nephrol.* (2020) 31:1128–39. doi: 10.1681/ASN.2019111168
61. McMurray JJV, Wheeler DC, Stefánsson BV, Jongs N, Postmus D, Correa-Rotter R, et al. Effect of dapagliflozin on clinical outcomes in patients with chronic kidney disease, with and without cardiovascular disease. *Circulation.* (2021) 2021:438–48. doi: 10.1161/CIRCULATIONAHA.120.051675
62. Chertow GM, Vart P, Jongs N, Toto RD, Gorriz JL, Hou FF, et al. Effects of dapagliflozin in stage 4 chronic kidney disease. *J Am Soc Nephrol.* (2021) 32:2352–61. doi: 10.1681/ASN.2021020167
63. Wheeler DC, Toto RD, Stefánsson B V, Jongs N, Chertow GM, Greene T, et al. A pre-specified analysis of the DAPA-CKD trial demonstrates the effects of dapagliflozin on major adverse kidney events in patients with IgA nephropathy. *Kidney Int.* (2021) 100:215–24. doi: 10.1016/j.kint.2021.03.033
64. Lee JF, Berzan E, Sridhar VS, Odutayo A, Cherney DZI. Cardiorenal protection in diabetic kidney disease. *Endocrinol Metab.* (2021) 36:256–69. doi: 10.3803/EnM.2021.987

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

MINI REVIEW
published: 06 January 2022
doi: 10.3389/fmed.2021.793990

Low Birthweight as a Risk Factor for Non-communicable Diseases in Adults

Maria Eugenia Bianchi[1*†] *and Jaime M. Restrepo*[2,3†]

[1] Laboratory Physiology, Department Basic Sciences, Institute School of Medicine, National Northeast University, Corrientes, Argentina, [2] Department of Pediatrics, Pediatric Nephrology Service, Icesi University, Valle del Lili, Cali, Colombia, [3] Fundación Valle del Lili, Cali, Colombia

According to studies undertaken over the past 40 years, low birthweight (LBW) is not only a significant predictor of perinatal death and morbidity, but also increases the risk of chronic non-communicable diseases (NCDs) in adulthood. The purpose of this paper is to summarize the research on LBW as a risk factor for NCDs in adults. The Barker hypothesis was based on the finding that adults with an LBW or an unhealthy intrauterine environment, as well as a rapid catch-up, die due to NCDs. Over the last few decades, terminology such as thrifty genes, fetal programming, developmental origins of health and disease (DOHaD), and epigenetic factors have been coined. The most common NCDs include cardiovascular disease, diabetes mellitus type 2 (DMT2), hypertension (HT), dyslipidemia, proteinuria, and chronic kidney disease (CKD). Studies in mothers who experienced famine and those that solely reported birth weight as a risk factor for mortality support the concept. Although the etiology of NCD is unknown, Barry Brenner explained the notion of a low glomerular number (nGlom) in LBW children, followed by the progression to hyperfiltration as the physiopathologic etiology of HT and CKD in adults based on Guyton's renal physiology work. Autopsies of several ethnic groups have revealed anatomopathologic evidence in fetuses and adult kidneys. Because of the renal reserve, demonstrating renal function in proportion to renal volume *in vivo* is more difficult in adults. The greatest impact of these theories can be seen in pediatrics and obstetrics practice.

Keywords: LBW (low birth weight), non-communicable diseases (NCDs) and risk factors, Barker hypothesis, CKD (chronic kidney disease), glomerular number

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Maria Goretti Moreira Guimarães Penido, Pediatric Nephrology Unit of Santa Casa de Belo Horizonte Hospital, Brazil

***Correspondence:**
Maria Eugenia Bianchi
mbianchi@med.unne.edu.ar

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *12 October 2021*
Accepted: *08 December 2021*
Published: *06 January 2022*

Citation:
Bianchi ME and Restrepo JM (2022) Low Birthweight as a Risk Factor for Non-communicable Diseases in Adults. Front. Med. 8:793990. doi: 10.3389/fmed.2021.793990

INTRODUCTION

Low weight and height newborns (NBs) (either preterm or with intrauterine growth restriction) are currently considered a public health issue. Every year, 1.1 million NBs die as a result of preterm delivery problems (1). Low birthweight (LBW) is not only a significant predictor of perinatal mortality and morbidity, but also raises the risk of chronic non-communicable diseases (NCDs), such as diabetes and cardiovascular disease, occurring in adulthood, according to studies conducted over the last 40 years (2).

LBW was identified as a future risk factor for NCDs in adults by David Barker (June 1938- August 2013), an English epidemiologist (3). To do so, he looked at the most common causes of death and their link to birthweight in a sample of 5,543 men born

in Hertfordshire, England between 1911 and 1930, a population that transitioned from famine to being a pillar of industrial growth. Years later, based on studies in a Norwegian cohort population (4), he claimed that "children who develop coronary heart disease and type 2 diabetes grow slowly throughout fetal life and infancy but rapidly increase their body mass indices afterward." Therefore, the risk factor for NCD mortality is generated not only during pregnancy, but also during the first years of life in relation to a child's nutrition, his/her environment, and associated epigenetic factors (5).

The concept of the "thrift gene" emerges for fetuses that have not been fed throughout pregnancy, as well as the hypothesis of biological plasticity: not only the genotype, but also the environment in which a life develops, is crucial. As a result, the term "fetal programming" was coined (6).

The origin of diseases associated with lifestyle is determined at the time of fertilization, passing through the stages of embryo, fetus, NB, and the first years of life, which implies the importance of the mother's health during pregnancy, according to the theory of the developmental origin of health and disease (DOHaD). Nutritional variables, as mentioned by Barker, can affect the mother's and child's health, but other factors, such as ambient air pollution (7), infectious diseases, stress, and toxins can also have an impact (8).

Barker's idea is thus a philosophical vision with substantial epidemiological foundations. It is framed in evolutionary theories, relating them to those of the French biologist Jean- Baptiste Lamark (1744-1829), who proposed that human plasticity, like that of all living beings, is influenced by epigenetic variables, as well as Darwin's natural selection rules (9).

ADULTS WITH LBW DIE FROM A VARIETY OF CAUSES AND PREVIOUSLY COMPARED TO NORMAL BIRTHWEIGHT INFANTS

The so-called Barker hypothesis has been established in a number of closed communities over the last 40 years with regard to NCD mortality. The requirement for obtaining this proof is birthweight records, which, according to the WHO, are not documented in even 10% of all children born. In general, this project relates to industrialized countries, where the frequency of this condition is decreasing, highlighting the necessity of such registries in undeveloped nations where the prevalence is higher (10). Norwegian studies, for example, are very strong and based on the Norwegian Medical Birth Registry, which has been recording birthweights across the country since 1967, and the Norwegian Patient Registry, which has been recording diagnostic codes for all admissions and office visits since 2008 (11). Researchers are now looking for alternative data sources, such as records from indigenous communities in Australia, which have been kept since 1956 by a Catholic mission (12).

Cardiovascular etiology, arterial hypertension, diabetes mellitus, cardiometabolic syndrome (13), and stroke were among the causes of death documented in these adult populations (14). Wendy Hoy et al. added respiratory and infectious causes of mortality in a small cohort of aboriginal adults in Australia (12).

Some characteristics of these studies can be summarized as follows: (a) In most articles, the deaths of LBW children are compared to the mortality of normal birthweight children in some cross-sectional studies. There are no control groups available. (b) The questions that arise are how old or how long it takes for LBW children to develop NCDs, as well as whether there are any other epigenetic variables in their lives that we can prevent. (c) Another factor to consider is that, because these studies were conducted on small, closed populations and life expectancy is anticipated for a given country, life expectancy for the years in which individuals were born was not included as a variable to be considered.

NCD IN ADULTS BORN WITH LBW

Two large groups studies are identified.

a.) *Population studies related to intrauterine hunger*: The most well-known studies are those from the Netherlands (the Dutch famine) (15), Biafra (16), Austria (17), China (18), and Nigeria (19), which generally associate intrauterine hunger with the development of cardiometabolic syndrome in adulthood, in some cases showing that the risk factor (intrauterine hunger) was independent of the children's birth weight (20).

b.) *Population studies in which factors other than birthweight are not taken into account.*

Premature delivery, low birthweight, and being small for one's gestational age have all been linked to NCD in adulthood, according to a meta-analysis published recently. The following NCDs were included in the selection criteria for the systematic review stage: obesity, being overweight, adipose tissue, diabetes, insulin resistance, hyperglycemia, glucose intolerance, and metabolic syndrome. Of 8,580 articles, 28 met the inclusion criteria with sufficient data and were retrospective and prospective. Seventy-five percent included both sexes, while the others only contained men (12.5%) or women (12.5%) (21).

Another meta-analysis contains studies in which children were categorized based on their birthweight, which ranged from <2 to 4.5 kg; they have a higher risk of developing type 2 DBT, cardiovascular disease, and HT as their weight declines. They found that women are more likely to develop HT, and that the development of HT follows a J-shaped pattern (it is observed in LBW and children with high birthweight) (22).

The 1966 Northern Finland Birth Cohort examined the association between birthweight and blood pressure at 31 years old, and revealed that birthweight was inversely related to blood pressure, notably in men (23). A 1986 study of the Northern Finnish birth cohort found a similar rise in systolic blood pressure at age 16, particularly among girls (24).

In the Chinese population, through the Shanghai Women's Health Study (SWHS) and the Shanghai Men's Health Study (SMHS), which recruited more than 100,000 people, it was shown

that LBW is an important risk factor for the development of obesity, diabetes, and hypertension in adulthood (25).

More than 150,000 women were enrolled in the Nurses' Health Study II (NHS II), which demonstrated that women with LBW have a higher risk of hypertension (26). The Bogalusa heart study also found that low birthweight is linked to systolic blood pressure fluctuation (27).

The limitations of these studies were: a) the researchers obtained significant results after adjusting for body size; b) various lifestyle factors—such as physical activity, smoking status, alcohol intake, family history, and socioeconomic status—were not considered in the evaluation of these relationships. Furthermore, in all populations around the world, females and males have different birthweights, so the association between birthweight and adult disease must be assessed separately for females and males (28).

POSSIBLE CAUSES FOR THE APPEARANCE OF NCDs IN ADULTS

On the one hand, the paradigmatic shift that Barker's concept entailed aimed to determine the causes of death, while on the other hand, the mechanisms by which epigenetic variables could increase the risk of HT, DMT2, and obesity in adults. The following can be mentioned:

1. Hormonal effects of the hypothalamic-pituitary axis, glucocorticoids, and growth hormone (29) act on the cardiovascular system or predispose patients to the development of obesity (8).
2. Epigenetics indicates something that we do not have in our genes, but which we can still pass on to our children. Epigenetics deals with changes in gene expression not resulting directly from mutations of DNA sequences, which lead to the formation of inherited traits both intra-generationally and inter-generationally. (30). Altered ketogenesis could be involved in the pathogenesis of DOHaD (31) through post-translational modification (32).
3. Studies that compare and contrast genetic and epigenetic effects. Mendelian randomization appears to be a new tool used in studies. The Nord-Trondelag Health (HUNT) Study is a population study that began in 1984 and involves the voluntary participation of individuals in clinical, phenotypic, and genotypic investigations (33, 34). There are Australian-Norwegian-British studies that caution about the interpretation of results deriving from Mendelian randomization (35).
4. Number of operational units: Reduced number of nephrons. Regarding the molecular mechanisms of nephron number reduction, the authors should mention oxidative stress, alterations in the renin-angiotensin system, alterations in sodium transporters, renal sympathetic activity, and the glucocorticoid effect.

Brenner et al. proposed in the 1980s (36) that in utero growth restriction results in a low nephron number, which may predispose patients to HT and kidney disease through mechanisms such as increased single nephron glomerular filtration, compensatory nephron hypertrophy, and decreased functional reserve. Approximately 60% of nephrons grow during the third trimester of pregnancy, and kidney development stops between 35 and 36 weeks of pregnancy (37). Thus, preterm delivery or delayed intrauterine growth may have a significant impact on nephron formation and nephron number (38).

Based on Guyton's basic science, physiology, and a positivist perspective, Barry Brenner and Valerie Luyck compiled reports on the consequences of LBW in the development of chronic kidney disease (CKD) (39–42).

The National Institute of Nephrology, the National Referral Center for Pathology, from Havana, Cuba, under the authorship of Reinaldo Maalich et al. demonstrated that the number of nephrons was decreased in dead fetuses, which was a paradigmatic leap from Brenner's hypothesis (43).

The lower number of nephrons would not only respond to nutritional aspects during pregnancy, but also highlight other causes, most of which mainly affect disadvantaged populations: pre-eclampsia, diabetes in gestation, maternal overweight/obesity, maternal underweight, advanced maternal age, adolescent pregnancy, and assisted reproduction. malaria during pregnancy, maternal chronic illness, and childhood overweight (44).

We agree that historical pathologic kidney descriptions identified the loss of nephrons as a cause of CKD (45). In post-mortem studies, it has been observed that total nephron number varies up to 13-fold (46). Wendy Hoy et al. showed in human kidneys, from a series of multiracial autopsies, that with age, the glomerular number decreased, and within a patient, there was a five-fold variability in mean glomerular capacity and significant heterogeneity in individual glomerular sizes. Lower glomerular number, higher body size, hypertension, and being black were all linked to larger mean glomerular volume and increased variability of individual glomerular volume. Glomerular enlargement, intimal thickening, and higher rates of glomerulosclerosis were all signs of hypertension as people became older. In whites and aborigines, but not in blacks in the US, a lower glomerular number was associated with hypertension (47).

Other studies were carried out by comparing the results of biopsies from living kidney donors associated with angiograms in the search for techniques that indicate glomerular number (48).

Imaging studies measuring renal volume were accepted to demonstrate the glomerular number *in vivo*, reverting to Dinkel's formula (49).

The most accessible were those of ultrasound, which gave rise to studies that entailed kidney measurements according to age and sex in each month and year in pediatrics (50), although not always specifying in the study sample the variables that would refer to nutritional status, blood pressure, and proteinuria of the healthy children (51, 52).

To determine kidney volume, magnetic resonance imaging (MRI) investigations are more accurate. These study lines were developed as a result of the experiences of polycystic kidney

research organizations (53). Recently, a technique for measuring *in vivo* was developed, which will once again open areas of inquiry into the demonstration of Brenner's theory, such as knowing the metabolism of a fetus' cells, despite all the constraints (54).

Renal volume, as gauged by the number of nephrons, and the existence of hypertension with glomerulomegaly have already been demonstrated, but due to the kidneys' renal reserve capacity, it is much more difficult to link renal size or nephron mass (55) to renal function. New study avenues were opened to explain why renal failure or arterial hypertension were not found in persons with a single kidney, as well as in transplant donors with fewer nephrons. This disease would not arise in people with single kidneys, whether transplanted or living donors, because nephron loss must occur during a key phase (when nephrogenesis is underway), implying that the quality of remaining nephrons is more essential than the quantity (11).

In a recent survey, 2,663,010 people were involved, and after a mean follow-up of 26 years (maximum 50 years), the odds ratio (OR) for CKD was 1.72 (95% CI, 1.60 −1.90), SGA was 1.79 (95% CI, 1.65–1.94), and preterm delivery was 1.48 (95% CI, 1.65–1.94). Analyses that used CKD diagnosis at stages 3–5 as an end point yielded similar results (56).

Recently, a population-based study (not a closed one) in Australia, with a total of 4,502 individuals who reported information about their birthweight, showed that birthweight had a positive association with eGFR (28).

Therefore, the Low Birthweight and Nephron Number Working Group has recommended that people with LBW be examined and followed up with to detect kidney disease or risk factors for renal illness at a young age (57).

IMPACT OF LBW IN PEDIATRICS AND OBSTETRICS

Based on these convincing works, the care of mothers and children during the first years of life has impacted political decisions around the world (58).

Each year, an estimated 13.7 million full-term children with LBW are born around the world, accounting for 11% of all births in poor nations. These children account for 75% of NB mortalities in Latin America (59).

Caroline Abitbol et al. in Miami found a link between the appearance of proteinuria and its subsequent increase, as measured by the protein/creatinine ratio in urine, and a subsequent deterioration of glomerular filtration ($r = 0.8$, $p = 0.0001$) and a tendency toward obesity with a body mass index (BMI) greater than the 85th percentile (89 percent sensitivity, PPV: 67%, $p = 0.03$) in newborns under 1,000 g. A subsequent study by the same center looked at the effects of obesity and prematurity on renal disease progression in a retrospective cohort of these children (44 obese and 36 non-obese). When compared to obese children born at term, patients who became obese after being born extremely preterm had a higher risk of impaired kidney function during childhood (hazard ratio 2.4; 95% CI: 1.1–7.1; $p = 0.04$) (60).

The foregoing becomes one of the main objectives in the postnatal monitoring of this group of children, as it is to monitor adequate weight gain during their weight-bearing growth to avoid excess protein intake, and therefore limit secondary hyperfiltration (61).

In a striking work done in Romania, they explored whether patterns of catch-up growth affect metabolic and cardiovascular outcomes in previously institutionalized adolescents. Children who are placed for adoption have higher levels of inflammation and HbA1c than those who remain in institutions. They emphasize in the discussion that adoptive parents should be informed about the potential consequences of shifting from perinatal restricted growth and rapid weight gain in infancy, plan a balanced diet with adequate nutrition and limiting obesogenic foods, plan regular physical activities, and begin these preventive measures with their children as soon as possible (62). They do not include qualitative variables such as less stress in their new homes.

CONCLUSIONS

The Barker hypothesis has strong connotations in the field of epidemiology from an evolutionary and mechanistic basis. The Brenner hypothesis is rooted in physiology and is positivistic. Thus, the adult patient whom we receive in the office with diabetes mellitus, obesity, HT, proteinuria, or CKD is the result of genetic and epigenetic conditions.

Maternal risks, such as overnutrition or undernutrition and GDM, which lead to low birthweight or high birthweight, certainly increase the risk for obesity and hypertension later in life as NCDs. Interventions in pre-conception or pre-pregnancy, as healthy habits, could be extremely important to reduce risks during pregnancy and the outcomes of childhood. Additionally, rapid changes in the environment, such as rapid urbanization, migration and new lifestyles, put these populations at higher risk of developing NCDs, especially in low- and middle-income countries.

In accordance with the DOHaD concept, adaptations occurring after environmental alterations would be advantageous in terms of population survival, when these alterations are consistent over several generations. However, they must be handled properly from a prevention and follow-up point of view.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. Lubchenco LO, Searls DT, Brazie JV. Neonatal mortality rate: relationship to birth weight and gestational age. *J Pediatr.* (1972) 81:814–22. doi: 10.1016/S0022-3476(72)80114-8
2. *World Health Organization*. Global Nutrition Targets 2025: Low Birth Weight Policy Brief. Geneva: World Health Organization (2014). Available online at: https://apps.who.int/iris/handle/10665/149020 (accessed October 2, 2021).
3. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet.* (1989) 2:1335.
4. Barker DJ, Osmond C, Kajantie E, Eriksson JG. Growth and chronic disease: findings in the Helsinki Birth Cohort. *Ann Hum Biol.* (2009) 36:445–58. doi: 10.1080/03014460902980295
5. Doom JR, Reid BM, Blanco E, Burrows R, Lozoff B, Gahagan S. Infant psychosocial environment predicts adolescent cardiometabolic risk: a prospective study. *J Pediatr.* (2019) 209:85–9. doi: 10.1016/j.jpeds.2019.01.058
6. Moreno-Fernandez J, Ochoa JJ, Lopez-Frias M, Diaz-Castro J. Impact of early nutrition, physical activity and sleep on the foetal programming of disease in the pregnancy: a narrative review. *Nutrients.* (2020) 20:3900. doi: 10.3390/nu12123900
7. Sarizadeh R, Dastoorpoor M, Goudarzi G, Simbar M. The association between air pollution and low birth weight and preterm labor in Ahvaz, Iran. *Int J Women's Health.* (2020) 12:313–25. doi: 10.2147/IJWH.S227049
8. Arima Y, Fukuoka H. Developmental origins of health and disease theory in cardiology. *J Cardiol.* (2020) 76:14–7. doi: 10.1016/j.jjcc.2020.02.003
9. John RM, Rougeulle C. Developmental epigenetics: phenotype and the flexible epigenome. *Front Cell Dev Biol.* (2018) 6:130. doi: 10.3389/fcell.2018.00130
10. Marete I, Ekhaguere O, Bann CM, Bucher SL, Nyongesa P, Patel AB, et al. Regional trends in birth weight in low- and middle-income countries 2013-2018. *Reprod Health.* (2020) 17:176. doi: 10.1186/s12978-020-01026-2
11. Gjerde A, Reisæter AV, Skrunes R, Marti HP, Vikse BE. Intrauterine growth restriction and risk of diverse forms of kidney disease during the first 50 years of life. *Clin J Am Soc Nephrol.* (2020) 15:1413–23. doi: 10.2215/CJN.04080320
12. Hoy WE, Nicol JL. The Barker hypothesis confirmed: association of low birth weight with all-cause natural deaths in young adult life in a remote Australian Aboriginal community. *J Dev Orig Health Dis.* (2019) 10:55–62. doi: 10.1017/S2040174417000903
13. Cooper, C. David Barker (1938–2013). *Nature.* (2013) 502:304. doi: 10.1038/502304a
14. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* (2016) 388:1659–724. doi: 10.1016/S0140-6736(16)31679-8
15. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev.* (2006) 82:485–91. doi: 10.1016/j.earlhumdev.2006.07.001
16. Hult M, Tornhammar P, Ueda P, Chima C, Bonamy AK, Ozumba B, Norman M. Hypertension, diabetes and overweight: looming legacies of the Biafran famine. *PLoS ONE.* (2010)5:e13582. doi: 10.1371/journal.pone.0013582
17. Thurner S, Klimek P, Szell M, Duftschmid G, Endel G, Kautzky-Willer A, et al. Quantification of excess risk for diabetes for those born in times of hunger, in an entire population of a nation, across a century. *Proc Natl Acad Sci U S A.* (2013) 110:4703–7. doi: 10.1073/pnas.1215626110
18. Zimmet P, Shi Z, El-Osta A, Ji L. Epidemic T2DM, early development and epigenetics: implications of the Chinese Famine. *Nat Rev Endocrinol.* (2018) 14:738–46. doi: 10.1038/s41574-018-0106-1
19. Loewenberg S. Famine fears in northeast Nigeria as Boko Haram fight rages. *Lancet.* (2017) 389:352. doi: 10.1016/S0140-6736(17)30198-8
20. Bleker LS, de Rooij SR, Painter RC, Ravelli AC, Roseboom TJ. Cohort profile: the Dutch famine birth cohort (DFBC) a prospective birth cohort study in the Netherlands. *BMJ Open.* (2021) 11:e042078. doi: 10.1136/bmjopen-2020-042078
21. de Mendonça ELSS, de Lima Macêna M, Bueno NB, de Oliveira ACM, Mello CS. Premature birth, low birth weight, small for gestational age and chronic non- communicable diseases in adult life: a systematic review with meta-analysis. *Early Hum Dev.* (2020) 149:105154. doi: 10.1016/j.earlhumdev.2020.105154
22. Xia Q, Cai H, Xiang YB, Zhou P, Li H, Yang G, et al. Prospective cohort studies of birth weight and risk of obesity, diabetes, and hypertension in adulthood among the Chinese population. *J Diabetes.* (2019) 11:55–64. doi: 10.1111/1753-0407.12800
23. Järvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, et al. Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. *Hypertension.* (2004) 44:838–46. doi: 10.1161/01.HYP.0000148304.33869.ee
24. Tikanmaki M, Tammelin T, Vaarasmaki M, Sipola-Leppanen M, Miettola S, Pouta A, et al. Prenatal determinants of physical activity and cardiorespiratory fitness in adolescence–Northern Finland Birth Cohort 1986 study. *BMC Public Health.* (2017) 17:346. doi: 10.1186/s12889-017-4237-4
25. Knop MR, Geng TT, Gorny AW, Ding R, Li C, Ley SH, Huang T. Birth weight and risk of type 2 diabetes mellitus, cardiovascular disease, and hypertension in adults: a meta-analysis of 7 646 267 participants from 135 studies. *J Am Heart Assoc.* (2018) 7:e008870. doi: 10.1161/JAHA.118.008870
26. Curhan GC, Chertow GM, Willett WC, Spiegelman D, Colditz GA, Manson JE, et al. Birth weight and adult hypertension and obesity in women. *Circulation.* (1996) 94:1310–5. doi: 10.1161/01.CIR.94.6.1310
27. Chen W, Srinivasan SR, Yao L, Li S, Dasmahapatra P, Fernandez C, etal. Lowbirth weight is associated with higher blood pressure variability from childhood to young adulthood: the Bogalusa Heart Study. *Am J Epidemiol.* (2012) 176(Suppl. 7): S99–105. doi: 10.1093/aje/kws298
28. Huxley R, Neil A, Collins R. Unravelling the foetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? *Lancet.* (2002) 360:659–65. doi: 10.1016/S0140-6736(02)09834-3
29. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci.* (2009) 3:19. doi: 10.3389/neuro.08.019.2009
30. Golic M, Stojanovska V, Bendix I, Wehner A, Herse F, Haase N, et al. Diabetes mellitus in pregnancy leads to growth restriction and epigenetic modification of the Srebf2 gene in rat fetuses. *Hypertension.* (2018) 71:911–20. doi: 10.1161/HYPERTENSIONAHA.117.10782
31. Hawdon JM, Ward Platt MP, Aynsley-Green A. Patterns of metabolic adaptation for preterm and term infants in the first neonatal week. *Arch Dis Child.* (1992) 67:357–65. doi: 10.1136/adc.67.4_Spec_No.357
32. Evans DM, Moen GH, Hwang LD, Lawlor DA, Warrington NM. Elucidating the role of maternal environmental exposures on offspring health and disease using two-sample Mendelian randomization. *Int J Epidemiol.* (2019) 48:861–75. doi: 10.1093/ije/dyz019
33. Moen GH, Brumpton B, Willer C, Åsvold BO, Birkeland KI, Wang G, et al. Mendelian randomization study of maternal influences on birthweight and future cardiometabolic risk in the HUNT cohort. *Nat Commun.* (2020) 11:5404. doi: 10.1038/s41467-020-19257-z
34. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol.* (2003) 32:1–22. doi: 10.1093/ije/dyg070
35. D'Urso S, Wang G, Hwang LD, Moen GH, Warrington NM, Evans DM, et al. cautionary note on using Mendelian randomization to examine the Barker hypothesis and Developmental Origins of Health and Disease (DOHaD). *J Dev Orig Health Dis.* (2021) 12:688–93. doi: 10.1017/S2040174420001105
36. Bacchetta J, Harambat J, Dubourg L, Guy B, Liutkus A, Canterino I, et al. Both extrauterine and intrauterine growth restriction impair renal function in children born very preterm. *Kidney Int.* (2009) 76: 445–452. doi: 10.1038/ki.2009.201
37. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. *Br J Obstet Gynaecol.* (1992) 99: 296–301. doi: 10.1111/j.1471-0528.1992.tb13726.x
38. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens.* (1988) 1:335–47. doi: 10.1093/ajh/1.4.335
39. Lackland DT, Bendall HE, Osmond C, Egan BM, Barker DJ. Low birth weights contribute to high rates of early-onset chronic renal failure in the Southeastern United States. *Arch Intern Med.* (200) 160:1472–6. doi: 10.1001/archinte.160.10.1472

40. Garcia-Garcia G, Jha V, World Kidney Day Steering Committee. CKD in disadvantaged populations. *Kidney Int.* (2015) 87:251–3. doi: 10.1038/ki.2014.369
41. Guyton AC, Coleman TG, Young DB, Lohmeier TE, DeClue JW. Salt balance and long- term blood pressure control. *Annu Rev Med*. (1980) 31:15–27.
42. Mañalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. *Kidney Int.* (2000) 58:770–3. doi: 10.1046/j.1523-1755.2000.00225.x
43. Luyckx VA, Brenner BM. Clinical consequences of developmental programming of low nephron number. *Anat Rec (Hoboken).* (2020) 303:2613–31. doi: 10.1002/ar.24270
44. Dunnill MS, Halley W. Some observations on the quantitative anatomy of the kidney. *J Pathol.* (1973) 110:113–21. doi: 10.1002/path.1711100202
45. Bertram JF, Douglas-Denton RN, Diouf B, Hughson MD, Hoy WE. Human nephron number: implications for health and dis- ease. *Pediatr Nephrol.* (2011) 26:1529–33. doi: 10.1007/s00467-011-1843-8
46. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD. Nephron number, glomerular volume, renal disease and hypertension. *Curr Opin Nephrol Hypertens.* (2008) 17:258–65. doi: 10.1097/MNH.0b013e3282f9b1a5
47. Sasaki T, Tsuboi N, Kanzaki G, Haruhara K, Okabayashi Y, Koike K, et al. Biopsy-based estimation of total nephron number in Japanese living kidney donors. *Clin Exp Nephrol.* (2019) 23:629–37. doi: 10.1007/s10157-018-01686-2
48. Dinkel E, Ertel M, Dittrich M, Peters H, Berres M, Schulte-Wisserman H. Kidney size in childhood. Sonographical growth charts for kidney length and volume. *Pediatr Radiol.* (1985) 15:38–43. doi: 10.1007/BF02387851
49. Bianchi ME, Forlino D, Velasco GA, Rodriguez PO, López G, Cusumano AM. Renal length and volume prediction in healthy children. *Clin Nephrol.* (2020) 93:31–35. doi: 10.5414/CNP92S105
50. Shi B, Yang Y, Li SX, Ju H, Ren WD. Ultrasonographic renal volume in Chinese children: results of 1683 cases. *J Pediatr Surg.* (2015) 50:1914–8. doi: 10.1016/j.jpedsurg.2015.05.011
51. Torres-Canchala L, Rengifo M, Filler G, Arias JC, Ramirez O, Restrepo JM. Low agreement between kidney volume and kidney length z-scores. *Pediatr Nephrol.* (2021) 36:1525–32. doi: 10.1007/s00467-020-04836-5
52. Torres-Sánchez MJ, Ávila-Barranco E, Esteban de la Rosa RJ, Fernández-Castillo R, Esteban MA, Carrero JJ, et al. Grupo para el Estudio de la Enfermedad Poliquística Autosómica Dominante (GEEPAD). Relationship between renal function and renal volume in autosomal dominant polycystic kidney disease: cross- sectional study. *Rev Clin Esp (Barc).* (2016) 216:62–7. doi: 10.1016/j.rceng.2015.11.002
53. Martins JR, Haenni D, Bugarski M, Polesel M, Schuh C, Hall AM. Intravital kidney microscopy: entering a new era. *Kidney Int.* (2021) 100:527–35. doi: 10.1016/j.kint.2021.02.042
54. Luyckx VA, Brenner BM. The clinical importance of nephron mass. *J Am Soc Nephrol.* (2010) 21:898–910. doi: 10.1681/ASN.2009121248
55. Singh RR, Denton KM, Bertram JF, Jefferies AJ, Head GA, Lombardo P. Schneider- Kolsky M, Moritz KM. Development of cardiovascular disease due to renal insuficiency in male sheep following fetal unilateral nephrectomy. *J Hypertens.* (2009) 27:386–96. doi: 10.1097/HJH.0b013e32831bc778
56. Luyckx VA, Perico N, Somaschini M, Manfellotto D, Valensise H, Cetin I et al. Writing group of the low birth weight and nephron number working group: a developmental approach to the prevention of hypertension and kidney disease: a report from the low birth weight and nephron number working group. *Lancet.* (2017) 390:424–8. doi: 10.1016/S0140-6736(17)30576-7
57. *The Thirteenth General Programme of Work, 2019–2023, was approved by the Seventy-first World Health Assembly in resolution WHA71*. (2018). Available online at: https://apps.who.int/iris/bitstream/handle/10665/324775/WHO-PRP-18.1-eng.pdf (accessed October 3, 2021).
58. De Onis M, Blossner M and Villar. Levels and patterns of intrauterine growth retardation in developing countries. *Eur J Clin Nutr.* (1998) 52:S 5–15.
59. Abitbol CL, Chandar J, Rodríguez MM, Berho M, Seeherunvong W, Freundlich M, et al. Obesity and preterm birth: additive risks in the progression of kidney disease in children. *Pediatr Nephrol.* (2009) 24:1363–70. doi: 10.1007/s00467-009-1120-2
60. Juvet C, Simeoni U, Yzydorczyk C, Siddeek B, Armengaud JB, Nardou K, et al. Effect of early postnatal nutrition on chronic kidney disease and arterial hypertension in adulthood: a narrative review. *J Dev Orig Health Dis.* (2018) 9:598–614. doi: 10.1017/S2040174418000454
61. Global Nutrition Targets 2025. *Low Birth Weight Policy Brief*. Available online at: WHO_NMH_NHD_14.5_eng.pdf (accessed October 3, 2021).
62. Tang A, Slopen N, Nelson CA, Zeanah CH, Georgieff MK, Fox NA. Catch-up growth, metabolic, and cardiovascular risk in post-institutionalized Romanian adolescents. *Pediatr Res.* (2018) 84:842–8. doi: 10.1038/s41390-018-0196-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 24 January 2022
doi: 10.3389/fmed.2021.746886

Uric Acid and Impairment of Renal Function in Non-diabetic Hypertensive Patients

Yi-Hsin Hung[1], *Chin-Chou Huang*[2,3,4,5*], *Liang-Yu Lin*[3,4,5,6] *and Jaw-Wen Chen*[2,4,5,7]

[1] Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, [2] Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [3] School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [4] Institute of Pharmacology, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [5] Cardiovascular Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan, [6] Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [7] Healthcare and Service Center, Taipei Veterans General Hospital, Taipei, Taiwan

Hyperuricemia is a risk factor for renal impairment. However, investigations focusing on patients with hypertension are limited and inconsistent. A single-center prospective cohort study of 411 Han Chinese non-diabetic hypertensive patients was conducted in Taiwan. The mean age of the participants was 62.0 ±14.4 years. The baseline estimated glomerular filtration rate and uric acid level were 86 mL/min/1.73 m^2 and 6.2 mg/dL, respectively. All patients underwent serum biochemistry tests for creatinine levels every 3 months. Renal events were defined as >25% and >50% decline in estimated glomerular filtration rate. During an average follow-up period of 4.7 ± 2.9 years (median 4.0 years), a >25 and >50% decline in estimated glomerular filtration rate was noted in 52 and 11 patients, respectively. The multivariate Cox regression analysis revealed that a baseline uric acid level ≥8.0 mg/dL increased the risk of >25% decline (hazard ratio: 3.541; 95% confidence interval: 1.655–7.574, $P = 0.001$) and >50% decline (hazard ratio: 6.995; 95% confidence interval: 1.309–37.385, $P = 0.023$) in estimated glomerular filtration rate. Similarly, a baseline uric acid level ≥7.5 mg/dL was independently associated with >25% decline (hazard ratio: 2.789; 95% confidence interval: 1.399–5.560, $P = 0.004$) and >50% decline (hazard ratio: 6.653; 95% confidence interval: 1.395–31.737, $P = 0.017$). However, this was not demonstrated at baseline uric acid level ≥7.0 mg/dL. Our study suggests that hyperuricemia is an independent risk factor for the decline in renal function in patients with hypertension. Uric acid level ≥7.5 mg/dL may be considered as the optimal cutoff value for clinical practice in predicting the development of renal impairment.

Keywords: Chinese, hypertension, renal function, nephropathy, uric acid

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Changli Wei, Rush University, United States
Baocheng Chang, Tianjin Medical University, China

***Correspondence:**
Chin-Chou Huang
cchuang4@vghtpe.gov.tw; huangchinchou@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *25 July 2021*
Accepted: *15 December 2021*
Published: *24 January 2022*

Citation:
Hung Y-H, Huang C-C, Lin L-Y and Chen J-W (2022) Uric Acid and Impairment of Renal Function in Non-diabetic Hypertensive Patients. Front. Med. 8:746886. doi: 10.3389/fmed.2021.746886

INTRODUCTION

Hypertension is a leading cause of chronic kidney disease (CKD) (1, 2). In addition to achieving blood pressure (BP) control, it is important to identify other possible risk factors to delay the development and progression of CKD.

Previous epidemiological studies on the general population have indicated an independent effect of hyperuricemia on the risk of developing CKD (3, 4). Several studies have focused on different subpopulations, such as patients with diabetic nephropathy and IgA nephropathy (5–7). However,

evidence regarding the relationship between uric acid (UA) and renal outcomes in hypertensive patients is limited and inconsistent (8, 9).

The physicochemical definition of hyperuricemia is based on the solubility limit of UA in serum (10). On the other hand, the statistical definition proposed by the American College of Rheumatology is UA above the mean plus two standard deviations for the healthy population (11). Based on the above definition, there is no universally accepted threshold and several cutoff values have been suggested, for example, >7.7 mg/dL in men and >6.6 mg/dL in women, or >7.0 mg/dL in men and >6.0 mg/dL in women (10, 12).

The guidelines of the American College of Rheumatology and the European Alliance of Associations for Rheumatology both propose the goal of managing patients with gout. However, they do not directly address the impact of hyperuricemia on renal diseases and hypertensive patients (13, 14). In addition, there is no clear UA cutoff associated with the risk of renal impairment. Whether screening of UA levels in hypertensive patients provides information for predicting and preventing renal diseases requires further research.

The present study focused on non-diabetic hypertensive patients and investigated the relationship between baseline serum UA levels and decline in renal function. In addition, we aimed to assess the serum UA cutoff value for predicting CKD development.

MATERIALS AND METHODS

Participants

Han Chinese patients with hypertension were included in our study from February 2012 to January 2021. The inclusion criteria were as follows: patients aged ≥20 years; those of Han Chinese descent; those who are official residents in Taiwan; those meeting one of the following hypertension criteria: (a) systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg in at least two consecutive visits within 2 months and (b) taking one or more antihypertensive medications; those with no medical history of severe diseases, including liver, renal, cardiac, and pulmonary failure and carcinoma; and those without acute disease within 2 weeks.

The exclusion criteria were as follows: the subject was identified as a secondary hypertension patient, unable to understand or give informed consent, and had one or more foreign parents. Patients with severe renal disease, defined as CKD stage 5 and end stage renal disease (ESRD), were excluded. Patients with diabetes mellitus and those who received uric acid-lowering agents within 3 months prior to the enrollment or during the study period were also excluded in the present study.

The study protocol was approved by the Ethics Committee of Taipei Veterans General Hospital. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Study Design

The study included a comprehensive evaluation of each participant's medical history and physical examination at the hypertension clinic of the hospital. The patients' office BP was measured, and their body mass indices (BMI) were determined. Antihypertensive drug prescriptions were recorded once they were present. All patients were followed up every 3 months.

Office BP Measurement

According to a standardized protocol, a well-trained nurse assessed the morning office BP using an electronic BP monitor (Omron HEM-7121, Omron Healthcare Taiwan Co., Songshan, Taipei, Taiwan, ROC) after the patients were instructed to sit for 10 min in a quiet room. During each measurement, both SBP and DBP were recorded. Three consecutive BP measurements were performed in the same upper arm. Each measurement was separated at an interval of 30 s. The average value of the last two measurements was considered the BP reading.

Laboratory Measurements

Fasting whole blood samples of the patients were obtained by venipuncture after a 10 min rest in a supine position in the morning, typically between 0730 and 0900 h. The participants were instructed to take all routine medications, as they normally would. The blood samples were centrifuged, and the serum was thawed for analysis. Serum levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), fasting blood glucose, creatinine, and UA were measured. Patients were further divided into different groups according to baseline UA levels (≥8.0, 7.5, or 7.0 mg/dL). Kidney function was assessed by serum creatinine at baseline and every 3 months thereafter. The estimated glomerular filtration rate (eGFR) was calculated using the four-variable equation proposed by the Modification of Diet in Renal Disease Study (15).

Renal Outcomes

Renal events during the follow-up period were defined as >25% decline or >50% decline in eGFR, which has been used to indicate minor or major renal dysfunction in previous studies (16, 17).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software (version 21.0, SPSS Inc., Chicago, IL, USA). All data are expressed as the mean ± standard deviation or frequency (percentage). Survival analysis was assessed using the Kaplan–Meier curve, with significance based on the log-rank test. To assess the independent effects of UA (baseline UA ≥8.0, 7.5, or 7.0 mg/dL) and renal outcomes, Cox proportional hazard regression analysis was performed. The adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated after adjusting for potential confounding factors, including age, sex, BMI, office SBP, use of antihypertensive drugs, use of furosemide, HDLC, and baseline eGFR. Statistical significance was defined as a two-sided $P < 0.05$.

RESULTS

The study enrolled 411 non-diabetic hypertensive participants in Taiwan. The mean age of the participants was 62.0 ± 14.4

TABLE 1 | Baseline characteristics.

	All ($n = 411$)		All ($n = 411$)
Age, years	62.0 ± 14.4	Aspirin, *n* (%)	41 (10.0%)
Male, *n* (%)	221 (53.8%)	Statins, *n* (%)	72 (17.6%)
BMI, kg/m^2	26.1 ± 3.9	Fibrate, *n* (%)	10 (2.4%)
Office SBP, mmHg	131.4 ± 16.9	Total cholesterol, mg/dL	188.1 ± 31.4
Office DBP, mmHg	81.6 ± 10.4	Triglyceride, mg/dL	128.8 ± 92.6
Office HR, bpm	70.8 ± 11.1	HDLC, mg/dL	49.4 ± 13.0
Smoking, *n* (%)	18 (4.4%)	LDLC, mg/dL	115.5 ± 27.4
ACEI/ARB, *n* (%)	264 (64.2%)	Fasting blood glucose, mg/dL	98.7 ± 12.5
β-blocker, *n* (%)	92 (22.4%)	UA, mg/dL	6.2 ± 1.5
CCB, *n* (%)	301 (73.2%)	Creatinine, mg/dL	0.9 ± 0.2
Thiazide, *n* (%)	87 (21.2%)	eGFR, mL/min /1.73 m^2	86.0 ± 19.4
Spironolactone, *n* (%)	6 (1.5%)	Follow-up duration, years	4.7 ± 2.9
Furosemide, *n* (%)	7 (1.7%)		

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, heart rate; LDLC, low density lipoprotein cholesterol; SBP, systolic blood pressure; UA, uric acid.

TABLE 2 | Baseline characteristics according to uric acid levels (≥8.0 mg/dL).

	UA <8.0 mg/dL ($n = 365$)	UA ≥8.0 mg/dL ($n = 46$)	*P*-value
Age, years	61.9 ± 13.9	62.5 ± 18.0	0.787
Male, *n* (%)	188 (51.5%)	33 (71.7%)	0.009
BMI, kg/m^2	26.0 ± 3.8	26.8 ± 4.6	0.264
Office SBP, mmHg	131.1 ± 16.6	133.8 ± 19.3	0.370
Office DBP, mmHg	81.8 ± 10.4	80.5 ± 10.4	0.428
Office HR, bpm	71.1 ± 11.1	68.3 ± 10.6	0.093
Smoking, *n* (%)	14 (3.8%)	4 (8.7%)	0.130
ACEI/ARB, *n* (%)	229 (62.7%)	35 (76.1%)	0.075
β-blocker, *n* (%)	82 (22.5%)	10 (21.7%)	0.911
CCB, *n* (%)	267 (73.2%)	34 (73.9%)	0.912
Thiazide, *n* (%)	70 (19.2%)	17 (37.0%)	0.005
Spironolactone, *n* (%)	5 (1.4%)	1 (2.2%)	0.512
Furosemide, *n* (%)	4 (1.1%)	3 (6.5%)	0.033
Aspirin, *n* (%)	35 (9.6%)	6 (13.0%)	0.437
Statins, *n* (%)	67 (18.4%)	5 (10.9%)	0.206
Fibrate, *n* (%)	9 (2.5%)	1 (2.2%)	>0.999
Total cholesterol, mg/dL	188.4 ± 31.8	186.4 ± 28.7	0.667
Triglyceride, mg/dL	125.3 ± 89.3	156.6 ± 112.7	0.075
HDLC, mg/dL	50.0 ± 13.4	44.7 ± 7.2	0.008
LDLC, mg/dL	115.7 ± 27.6	114.0 ± 25.7	0.681
Fasting blood glucose, mg/dL	98.7 ± 12.7	98.5 ± 10.8	0.934
UA, mg/dL	5.8 ± 1.1	9.1 ± 0.8	<0.001
Creatinine, mg/dL	0.8 ± 0.2	1.0 ± 0.3	<0.001
eGFR, mL/min /1.73 m^2	87.3 ± 18.8	76.0 ± 20.7	0.001
Follow-up duration, years	4.7 ± 2.9	4.9 ± 2.8	0.557

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, heart rate; LDLC, low density lipoprotein cholesterol; SBP, systolic blood pressure; UA, uric acid.

years, and 53.8% were men. There were 4.4% of the participants being smokers. The mean BMI was 26.1 ± 3.9 kg/m^2. The mean office SBP and DBP were 131.4 ± 16.9 and 81.6 ± 10.4 mmHg, respectively. The baseline UA level was 6.2 ± 1.5 mg/dL. The renal function of the participants upon enrollment was serum creatinine level of 0.9 ± 0.2 mg/dL and eGFR of 86.0 ± 19.4 mL/min/1.73 m^2. The lipid profiles were as the followings, mean total cholesterol being 188.1 ± 31.4 mg/dL, triglyceride being 128.8 ± 92.6 mg/dL, HDLC being 49.4 ± 13.0 mg/dL, and LDLC being 115.5 ± 27.4 mg/dL. The use of antihypertensive medications included angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs) (64.2%), β-blockers (22.4%), calcium channel blockers (73.2%), and thiazide (21.2%). The use of other diuretics included spironolactone (1.5%) and furosemide (1.7%). There were 10.0, 17.6, and 2.4% of the participants taking aspirin, statins and fibrate, respectively (**Table 1**).

When compared to those with lower baseline UA levels, patients with higher baseline UA levels were more likely to be male, have a higher BMI, have a worse renal function, have a lower HDLC, and use thiazide and furosemide (**Tables 2**–**4**).

During a mean follow-up period of 4.7 ± 2.9 years (median 4.0 years), a >25% and >50% decline in eGFR was noted in 52 and 11 patients, respectively. Participants with higher baseline UA levels had higher rates of renal events than their counterparts. A statistically significant increase in the incidence of >25% decline in eGFR was observed if the baseline UA was ≥8.0 mg/dL ($P = 0.004$) or ≥7.5 mg/dL ($P = 0.040$). Moreover, a statistically significant increase in the incidence of >50% decline in eGFR was observed if the baseline UA was ≥8.0 mg/dL ($P = 0.025$) or ≥7.5 mg/dL ($P = 0.035$) (**Table 5**).

The Kaplan–Meier survival curves and log-rank test were used to identify the number of participants who did not develop renal impairment during the follow-up period. The incidence of renal events (>25% and >50% decline in eGFR) was significantly higher in patients with a baseline UA level ≥8.0 mg/dL ($P = 0.033$ and 0.014, respectively) (**Figures 1A,B**). Similarly, the participants who presented with baseline UA ≥7.5 mg/dL had more renal events (>50% decline in eGFR) ($P = 0.022$) (**Figures 2A,B**). However, the participants who presented with baseline UA ≥7.0 mg/dL during the initial visit had similar renal events (>25% and >50% decline in eGFR) ($P = 0.673$ and 0.202, respectively) (**Figures 3A,B**).

Multivariate Cox regression analysis revealed that a baseline UA level ≥8.0 mg/dL was independently associated with a risk of >25% decline in eGFR (HR: 3.541; 95% CI: 1.655–7.574, $P = 0.001$) and a >50% decline in eGFR (HR: 6.995; 95% CI: 1.309–37.385, $P = 0.023$) (**Table 6**). Similarly, a baseline UA level ≥7.5 mg/dL was independently associated with a

TABLE 3 | Baseline characteristics according to uric acid level (≥7.5 mg/dL).

	UA <7.5 mg/dL (*n* = 335)	UA ≥7.5 mg/dL (*n* = 76)	*P*-value
Age, years	62.3 ± 13.8	60.5 ± 17.0	0.317
Male, *n* (%)	163 (48.7%)	58 (76.3%)	<0.001
BMI, kg/m^2	25.9 ± 3.8	27.2 ± 4.4	0.022
Office SBP, mmHg	131.1 ± 16.4	132.7 ± 18.9	0.481
Office DBP, mmHg	81.8 ± 10.2	80.8 ± 11.3	0.465
Office HR, bpm	70.8 ± 10.8	70.6 ± 12.2	0.885
Smoking, *n* (%)	13 (3.9%)	5 (6.6%)	0.347
ACEI/ARB, *n* (%)	208 (62.1%)	56 (73.7%)	0.057
β-blocker, *n* (%)	76 (22.7%)	16 (21.1%)	0.758
CCB, *n* (%)	245 (73.1%)	56 (73.7%)	0.922
Thiazide, *n* (%)	60 (17.9%)	27 (35.5%)	0.001
Spironolactone, *n* (%)	4 (1.2%)	2 (2.6%)	0.307
Furosemide, *n* (%)	4 (1.2%)	3 (3.9%)	0.121
Aspirin, *n* (%)	33 (9.9%)	8 (10.5%)	0.859
Statins, *n* (%)	64 (19.2%)	8 (10.5%)	0.074
Fibrate, *n* (%)	8 (2.4%)	2 (2.6%)	>0.999
Total cholesterol, mg/dL	188.2 ± 31.8	187.7 ± 29.6	0.881
Triglyceride, mg/dL	125.0 ± 91.8	145.7 ± 94.8	0.088
HDLC, mg/dL	50.5 ± 13.5	45.0 ± 8.8	0.001
LDLC, mg/dL	115.1 ± 27.6	117.1 ± 26.5	0.556
Fasting blood glucose, mg/dL	98.5 ± 12.5	99.3 ± 12.5	0.649
UA, mg/dL	5.7 ± 1.0	8.5 ± 1.0	<0.001
Creatinine, mg/dL	0.8 ± 0.2	1.0 ± 0.2	<0.001
eGFR, mL/min /1.73 m^2	87.7 ± 19.2	78.6 ± 18.4	<0.001
Follow-up duration, years	4.7 ± 2.9	4.7 ± 2.7	0.918

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, heart rate; LDLC, low density lipoprotein cholesterol; SBP, systolic blood pressure; UA, uric acid.

TABLE 4 | Baseline characteristics according to uric acid level (≥7.0 mg/dL).

	UA <7.0 mg/dL (*n* = 296)	UA ≥7.0 mg/dL (*n* = 115)	*P*-value
Age, years	62.3 ±13.6	61.1 ± 16.3	0.441
Male, *n* (%)	131 (44.3%)	90 (78.3%)	<0.001
BMI, kg/m^2	25.8 ± 3.7	27.0 ± 4.3	0.008
Office SBP, mmHg	130.6 ± 16.2	133.3 ± 18.4	0.173
Office DBP, mmHg	81.6 ± 10.2	81.6 ± 10.8	0.948
Office HR, bpm	70.6 ± 10.6	71.2 ± 12.3	0.693
Smoking, *n* (%)	12 (4.1%)	6 (5.2%)	0.605
ACEI/ARB, *n* (%)	178 (60.1%)	86 (74.8%)	0.005
β-blocker, *n* (%)	64 (21.6%)	28 (24.3%)	0.552
CCB, *n* (%)	216 (73.0%)	85 (73.9%)	0.847
Thiazide, *n* (%)	45 (15.2%)	42 (36.5%)	<0.001
Spironolactone, *n* (%)	4 (1.4%)	2 (1.7%)	0.674
Furosemide, *n* (%)	4 (1.4%)	3 (2.6%)	0.405
Aspirin, *n* (%)	26 (8.8%)	15 (13.0%)	0.196
Statins, *n* (%)	59 (20.0%)	13 (11.3%)	0.038
Fibrate, *n* (%)	8 (2.7%)	2 (1.7%)	0.732
Total cholesterol, mg/dL	186.7 ± 32.1	191.8 ± 29.3	0.125
Triglyceride, mg/dL	119.1 ± 80.4	153.7 ± 115.1	0.001
HDLC, mg/dL	51.0 ± 13.7	45.5 ± 9.9	<0.001
LDLC, mg/dL	114.2 ± 28.0	118.8 ± 25.5	0.110
Fasting blood glucose, mg/dL	97.8 ± 11.9	100.9 ± 13.7	0.023
UA, mg/dL	5.5 ± 0.9	8.1 ± 1.0	<0.001
Creatinine, mg/dL	0.8 ± 0.2	1.0 ± 0.2	<0.001
eGFR, mL/min /1.73 m^2	88.4 ± 19.5	79.8 ± 17.6	<0.001
Follow-up duration, years	4.7 ± 2.9	4.7 ± 2.8	0.836

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, heart rate; LDLC, low density lipoprotein cholesterol; SBP, systolic blood pressure; UA, uric acid.

>25% decline in eGFR (HR: 2.789; 95% CI: 1.399–5.560, $P = 0.004$) and >50% decline in eGFR (HR: 6.653; 95% CI: 1.395–31.737, $P = 0.017$) (**Table 7**). However, a baseline UA level ≥7.0 mg/dL was not associated with a >25% decline in eGFR (HR: 1.577; 95% CI: 0.803–3.095, $P = 0.186$) or >50% decline in eGFR (HR: 2.756; 95% CI: 0.607–12.519, $P = 0.189$) (**Table 8**).

The subgroup analysis by gender was further conducted. A baseline UA level ≥8.0 mg/dL was associated with a risk of >25% decline in eGFR in both female (HR: 5.658; 95% CI: 1.244–25.747, $P = 0.025$) and male (HR: 2.798; 95% CI: 1.147–6.825, $P = 0.024$). As we further lower the cut-off value, a baseline UA level ≥7.5 mg/dL was associated with a risk of >25% decline in eGFR in male (HR: 2.374; 95% CI: 1.013–5.559, $P = 0.047$), but not in female (HR: 3.454; 95% CI: 0.895–13.332, $P = 0.072$). As for the major renal event, a baseline UA level ≥8.0 mg/dL was associated with a >50% decline in eGFR in female (HR: 40.086; 95% CI: 2.606–616.712, $P = 0.008$), but not in male (HR: 7.320; 95% CI: 0.476–112.592, $P = 0.153$). However, *P*-values for interaction were all insignificant (**Table 9**).

TABLE 5 | Uric acid and renal events.

	Patient number	>25% decline in eGFR, *n* (%)	*P*-value	>50% decline in eGFR, *n* (%)	*P*-value
UA levels					
<8.0 mg/dL	365	40 (11.0%)	0.004	7 (1.9%)	0.025
≥8.0 mg/dL	46	12 (26.1%)		4 (8.7%)	
UA levels					
<7.5 mg/dL	335	37 (11.0%)	0.040	6 (1.8%)	0.035
≥7.5 mg/dL	76	15 (19.7%)		5 (6.6%)	
UA levels					
<7.0 mg/dL	296	35 (11.8%)	0.418	6 (2.0%)	0.191
≥7.0 mg/dL	115	17 (14.8%)		5 (4.3%)	

eGFR, estimated glomerular filtration rate; UA, uric acid.

DISCUSSION

This study aimed to investigate the relationship between baseline serum UA levels and renal outcomes in patients with

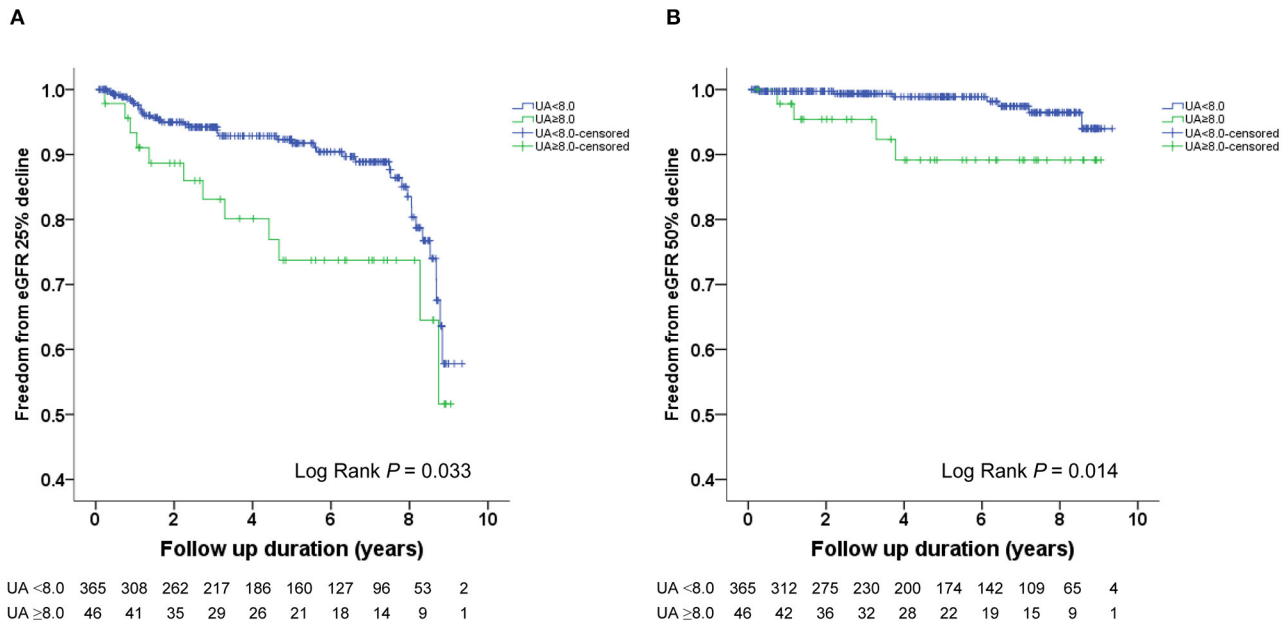


FIGURE 1 | Kaplan-Meier survival curves showing the absence of renal events according to the baseline uric acid (UA) in patients with hypertension. All participants were divided into two groups according to UA levels. The blue line represents the patient group with UA <8.0 mg/dL. The green line represents the group with UA ≥8.0 mg/dL. Renal events were defined as >25% decline and >50% decline in eGFR. Differences were compared using the log-rank test. **(A)** UA (<8.0 vs. ≥8.0 mg/dL) and >25% decline in eGFR ($P = 0.033$). **(B)** UA (<8.0 vs. ≥8.0 mg/dL) and >50% decline in eGFR ($P = 0.014$).

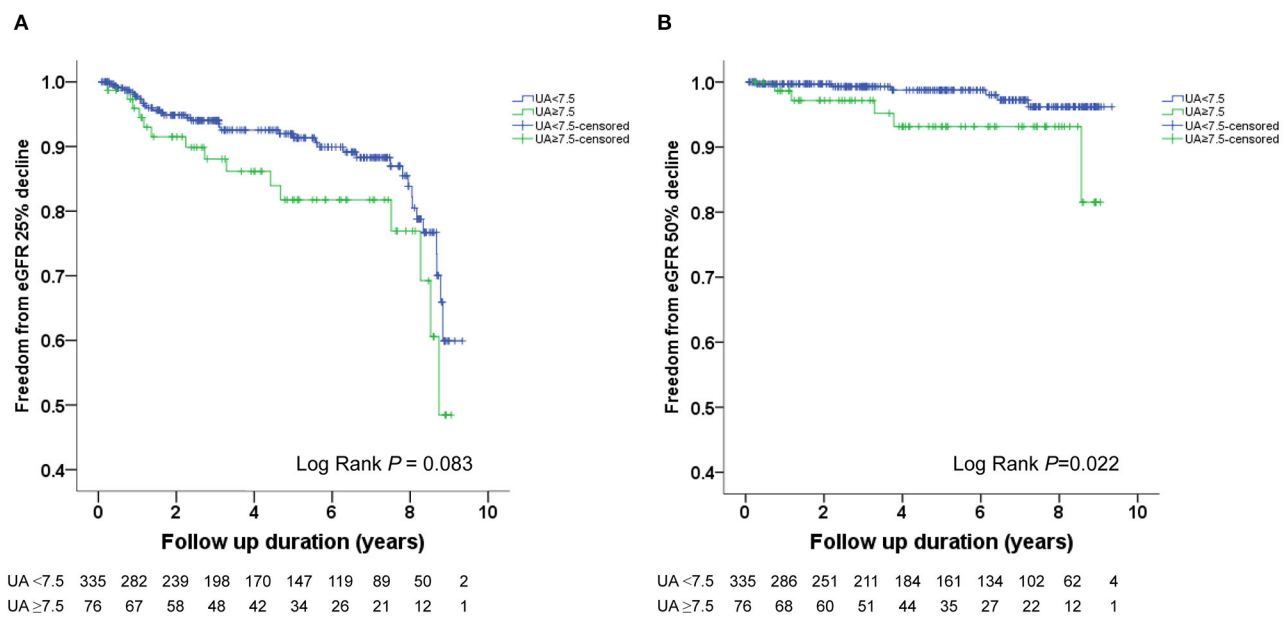


FIGURE 2 | Kaplan-Meier survival curves showing the absence of renal events according to the baseline uric acid (UA) in patients with hypertension. All participants were divided into two groups according to UA levels. The blue line represents the patient group with UA <7.5 mg/dL. The green line represents the group with UA ≥7.5 mg/dL. Renal events were defined as >25% decline and >50% decline in eGFR. Differences were compared using the log-rank test. **(A)** UA (<7.5 vs. ≥7.5 mg/dL) and >25% decline in eGFR ($P = 0.083$). **(B)** UA (<7.5 vs. ≥7.5 mg/dL) and >50% decline in eGFR ($P = 0.022$).

hypertension. Our investigation suggests that hyperuricemia, with a cutoff of 7.5 or 8.0 mg/dL, is related to the decline of renal function in Han Chinese hypertensive patients in Taiwan.

Several modifiable and unmodifiable mediators are related to the development and progression of CKD (18, 19). Among them, hypertension was one of the most important contributors

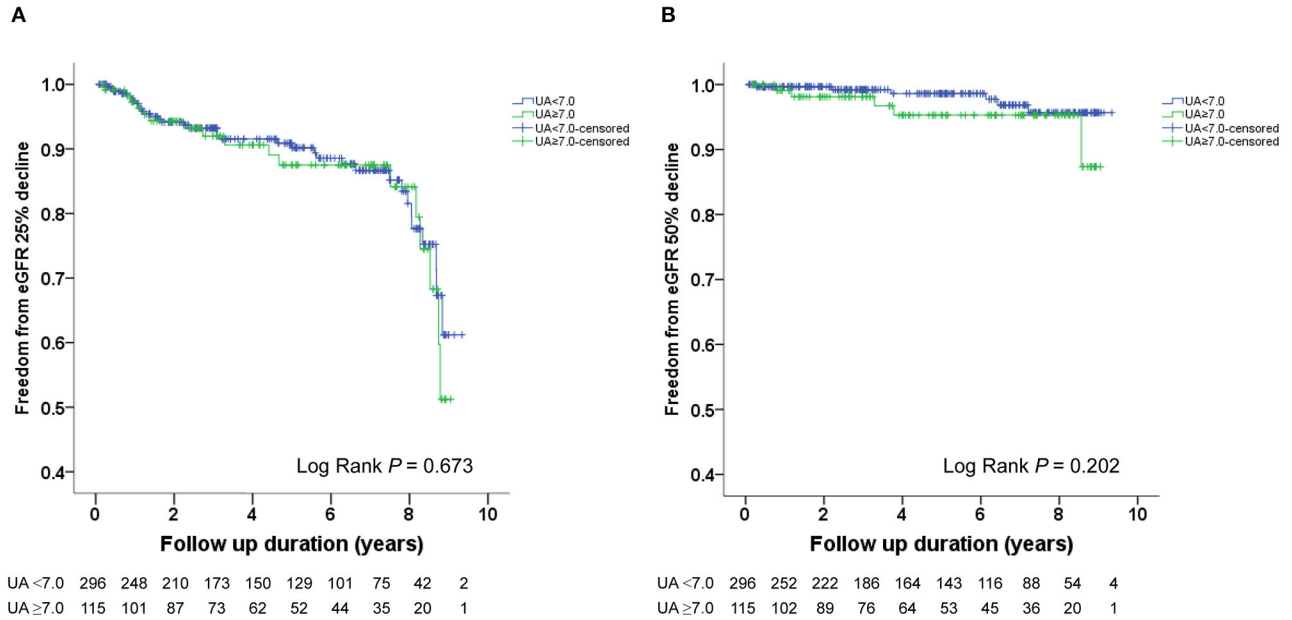


FIGURE 3 | Kaplan-Meier survival curves showing the absence of renal events according to the baseline uric acid (UA) in patients with hypertension. All participants were divided into two groups according to UA levels. The blue line represents the patient group with UA <7.0 mg/dL. The green line represents the group with UA ≥7.0 mg/dL. Renal events were defined as >25% decline and >50% decline in eGFR. Differences were compared using the log-rank test. **(A)** UA (<7.0 vs. ≥7.0 mg/dL) and >25% decline in eGFR ($P = 0.673$). **(B)** UA (<7.0 vs. ≥7.0 mg/dL) and >50% decline in eGFR ($P = 0.202$).

TABLE 6 | Uric acid 8.0 mg/dL and decline of estimated glomerular filtration rate (eGFR).

eGFR >25% reduction	HR	(95% CI)	*P*-value	eGFR >50% reduction	HR	(95% CI)	*P*-value
Univariate analysis				**Univariate analysis**			
UA ≥8.0 mg/dL (yes vs. no)	1.997	(1.043–3.820)	0.037	UA ≥8.0 mg/dL (yes vs. no)	4.151	(1.213–14.201)	0.023
Multivariate analysis				**Multivariate analysis**			
Age, years	1.036	(1.009–1.063)	0.007	Age, years	1.023	(0.959–1.091)	0.489
Sex (male vs. female)	0.738	(0.387–1.409)	0.357	Sex (male vs. female)	0.273	(0.063–1.186)	0.083
BMI, kg/m^2	0.998	(0.917–1.086)	0.957	BMI, kg/m^2	0.923	(0.755–1.128)	0.433
Office SBP, mmHg	1.021	(1.004–1.038)	0.017	Office SBP, mmHg	1.056	(1.014–1.099)	0.008
ACEI/ARB (yes vs. no)	0.572	(0.303–1.079)	0.085	ACEI/ARB (yes vs. no)	0.474	(0.106–2.122)	0.329
β-blocker (yes vs. no)	1.605	(0.873–2.952)	0.128	β-blocker (yes vs. no)	1.961	(0.474–8.125)	0.353
CCB (yes vs. no)	1.279	(0.638–2.562)	0.488	CCB (yes vs. no)	0.412	(0.096–1.765)	0.232
Thiazide (yes vs. no)	1.156	(0.597–2.238)	0.667	Thiazide (yes vs. no)	2.116	(0.541–8.274)	0.281
Furosemide (yes vs. no)	0.782	(0.097–6.289)	0.817	Furosemide (yes vs. no)	14.990	(1.021–220.070)	0.048
HDLC, mg/dL	0.988	(0.962–1.014)	0.365	HDLC, mg/dL	0.963	(0.904–1.026)	0.243
eGFR, mL/min /1.73 m^2	1.024	(1.007–1.042)	0.005	eGFR, mL/min /1.73 m^2	1.026	(0.990–1.064)	0.157
UA ≥8.0 mg/dL (yes vs. no)	3.541	(1.655–7.574)	0.001	UA ≥8.0 mg/dL (yes vs. no)	6.995	(1.309–37.385)	0.023

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, hazard ratio; SBP, systolic blood pressure; UA, uric acid.

to CKD (1, 2). In some hypertensive patients, however, renal function continued to deteriorate progressively even when the BP was under control. In our previous study, 11.2% of hypertensive patients still suffered from renal function decline when their BP was controlled to <140/90 mmHg (17). It is essential to identify specific characteristics that increase the risk of renal insufficiency in this population. Therefore, we focused on another possible modifiable risk factor, hyperuricemia.

Several studies have indicated that hyperuricemia is a predictor of the occurrence of renal disease in the general population. Two community cohorts in the United States, which involved 13,338 participants with 8.5 years of follow-up, suggested that elevated UA levels were an independent risk

TABLE 7 | Uric acid 7.5 mg/dL and decline of estimated glomerular filtration rate (eGFR).

eGFR >25% reduction	HR	(95% CI)	*P*-value	eGFR >50% reduction	HR	(95% CI)	*P*-value
Univariate analysis				**Univariate analysis**			
UA ≥7.5 mg/dL (yes vs. no)	1.690	(0.927–3.081)	0.087	UA ≥7.5 mg/dL (yes vs. no)	3.658	(1.115–12.002)	0.032
Multivariate analysis				**Multivariate analysis**			
Age, years	1.036	(1.009–1.064)	0.008	Age, years	1.024	(0.960–1.092)	0.473
Sex (male vs. female)	0.724	(0.383–1.369)	0.320	Sex (male vs. female)	0.273	(0.066–1.136)	0.074
BMI, kg/m^2	0.993	(0.911–1.083)	0.879	BMI, kg/m^2	0.897	(0.731–1.100)	0.297
Office SBP, mmHg	1.021	(1.004–1.038)	0.017	Office SBP, mmHg	1.057	(1.013–1.102)	0.010
ACEI/ARB (yes vs. no)	0.600	(0.319–1.128)	0.113	ACEI/ARB (yes vs. no)	0.491	(0.109–2.206)	0.354
β-blocker (yes vs. no)	1.574	(0.861–2.878)	0.140	β-blocker (yes vs. no)	2.071	(0.491–8.739)	0.322
CCB (yes vs. no)	1.271	(0.637–2.535)	0.496	CCB (yes vs. no)	0.412	(0.097–1.747)	0.229
Thiazide (yes vs. no)	1.100	(0.563–2.151)	0.780	Thiazide (yes vs. no)	1.926	(0.474–7.826)	0.360
Furosemide (yes vs. no)	0.850	(0.106–6.822)	0.878	Furosemide (yes vs. no)	19.968	(1.274–312.928)	0.033
HDLC, mg/dL	0.986	(0.961–1.013)	0.314	HDLC, mg/dL	0.961	(0.902–1.024)	0.217
eGFR, mL/min /1.73 m^2	1.023	(1.006–1.041)	0.008	eGFR, mL/min /1.73 m^2	1.026	(0.990–1.063)	0.161
UA ≥7.5 mg/dL (yes vs. no)	2.789	(1.399–5.560)	0.004	UA ≥7.5 mg/dL (yes vs. no)	6.653	(1.395–31.737)	0.017

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, hazard ratio; SBP, systolic blood pressure; UA, uric acid.

TABLE 8 | Uric acid 7.0 mg/dL and decline of estimated glomerular filtration rate (eGFR).

eGFR >25% reduction	HR	(95% CI)	*P*-value	eGFR >50% reduction	HR	(95% CI)	*P*-value
Univariate analysis				**Univariate analysis**			
UA ≥7.0 mg/dL (yes vs. no)	1.133	(0.634–2.023)	0.673	UA ≥7.0 mg/dL (yes vs. no)	2.126	(0.649–6.969)	0.213
Multivariate analysis				**Multivariate analysis**			
Age, years	1.033	(1.006–1.060)	0.018	Age, years	1.018	(0.956–1.083)	0.584
Sex (male vs. female)	0.732	(0.382–1.403)	0.347	Sex (male vs. female)	0.298	(0.071–1.252)	0.098
BMI, kg/m^2	0.996	(0.913–1.087)	0.929	BMI, kg/m^2	0.904	(0.738–1.108)	0.330
Office SBP, mmHg	1.021	(1.004–1.039)	0.015	Office SBP, mmHg	1.055	(1.013–1.098)	0.009
ACEI/ARB (yes vs. no)	0.648	(0.347–1.209)	0.173	ACEI/ARB (yes vs. no)	0.601	(0.141–2.572)	0.493
β-blocker (yes vs. no)	1.451	(0.797–2.640)	0.223	β-blocker (yes vs. no)	1.581	(0.403–6.206)	0.511
CCB (yes vs. no)	1.303	(0.657–2.584)	0.449	CCB (yes vs. no)	0.513	(0.130–2.021)	0.340
Thiazide (yes vs. no)	1.080	(0.540–2.160)	0.829	Thiazide (yes vs. no)	1.847	(0.442–7.720)	0.400
Furosemide (yes vs. no)	0.893	(0.111–7.156)	0.915	Furosemide (yes vs. no)	16.980	(1.243–232.037)	0.034
HDLC, mg/dL	0.985	(0.959–1.011)	0.259	HDLC, mg/dL	0.961	(0.905–1.021)	0.195
eGFR, mL/min /1.73 m^2	1.019	(1.001–1.036)	0.034	eGFR, mL/min /1.73 m^2	1.017	(0.981–1.054)	0.354
UA ≥7.0 mg/dL	1.577	(0.803–3.095)	0.186	UA ≥7.0 mg/dL	2.756	(0.607–12.519)	0.189

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HDLC, high density lipoprotein cholesterol; HR, hazard ratio; SBP, systolic blood pressure; UA, uric acid.

factor for incident kidney disease (3). Another mass community-based screening conducted in Japan, with 48,177 participants, further identified UA level as a major factor for ESRD in females during 7 years. The study showed that the incidence of ESRD per 1,000 women was 0.87 for those without hyperuricemia and 9.03 for those with hyperuricemia, with a hazard ratio of 5.77 (4).

In addition to the development of kidney diseases, high UA levels have been shown to exacerbate the progression of renal impairment, including diabetic nephropathy (5–7), IgA nephropathy (20–22), nephrosclerosis (23), and allograft nephropathy (24, 25). However, few studies have focused on patients with hypertension. The Uric Acid Right for Heart Health (URRAH) project, a cross-sectional study with 26,971 Italian patients with 62% being hypertensive patients, indicated that those with CKD were 10 times more likely to have hyperuricemia than those with intact renal function (8). Whether hyperuricemia presented as the cause, co-existing factor, or consequence of CKD was not investigated in this observational study. On the other hand, a 4.8-year cohort study in Japan demonstrated that UA level was not an independent risk factor for ESRD in hypertensive nephropathy (9). However, the follow-up period might be insufficient for progression to ESRD, making the results unremarkable. On the contrary, our prospective

TABLE 9 | Uric acid levels and decline of estimated glomerular filtration rate (eGFR) in female and male.

eGFR25% decline	Female			Male			
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	*P* for interaction
UA ≥8.0 mg/dL (yes vs. no)*	5.658	(1.244–25.747)	0.025	2.798	(1.147–6.825)	0.024	0.373
UA ≥7.5 mg/dL (yes vs. no)*	3.454	(0.895–13.332)	0.072	2.374	(1.013–5.559)	0.047	0.381
UA ≥7.0 mg/dL (yes vs. no)*	1.792	(0.472–6.800)	0.391	1.290	(0.549–3.032)	0.559	0.461
eGFR50% decline	**Female**			**Male**			
	HR	(95% CI)	*P*-value	HR	(95% CI)	*P*-value	*P* for interaction
UA ≥8.0 mg/dL (yes vs. no)*	40.086	(2.606–616.712)	0.008	7.320	(0.476–112.592)	0.153	0.681
UA ≥7.5 mg/dL (yes vs. no)*	7.269	(0.758–69.757)	0.086	308.437	(0.233–407815.643)	0.118	0.614
UA ≥7.0 mg/dL (yes vs. no)*	4.427	(0.422–46.401)	0.215	24.980	(0.761–820.370)	0.071	0.809

CI, confidence interval; HR, hazard ratio; UA, uric acid.

**Adjusted for age, BMI, office SBP, ACEI/ARB, β-blocker, CCB, thiazide, furosemide, HDLC, and eGFR.*

study emphasized early prevention of CKD in hypertensive patients by defining renal events as >25% and >50% reduction in eGFR.

Despite the strong association referred to by the above epidemiological data, the precise pathogenetic mechanism for urate nephropathy has not been well-established. It was hypothesized that the deposition of urate crystals in the medullary interstitium induced an inflammatory response, potentially leading to interstitial fibrosis and eventually CKD (26–28). The histological changes, including needle-like birefringent crystals of urate along with vascular sclerosis and tubular atrophy, provided evidence for urate nephropathy (29). However, both the pathological evidence and clinical manifestations were non-specific, making it difficult to differentiate it from other common etiologies, such as diabetic nephropathy. Whether hyperuricemia serves as a marker or contributor to renal injury is still under debate (30–32). To clarify the association between the UA level and renal outcome, we included patients with relatively preserved renal function at baseline (eGFR of 86.0 ± 19.4 mL/min/1.73 m^2) and excluded those with diabetes mellitus. Other possible causes that affected renal function, including smoking (33), metabolic syndrome (34), and use of fibrate, statin or other medication (35, 36) were analyzed as well. The significant results of our study implied that hyperuricemia contributes to renal impairment.

There is no consensus on the target UA level in either the general population or patients with hypertension. The American College of Rheumatology Guideline suggested initiating intervention in patients with first gout flare only when the UA level exceeds 9.0 mg/dL, targeting a UA level <6.5 mg/dL (13). The European Alliance of Associations for Rheumatology proposed a stricter goal with initiating treatment in those with UA >8.5 mg/dL and targeting a UA level <6.0 mg/dL (14). However, the above recommendation applies only to patients with gout and does not address the impact of hyperuricemia on renal disease or hypertensive patients. Several studies have aimed to provide cutoff values for the prediction of renal disease. A study conducted in Vienna, with 21,475 healthy volunteers and a 7-year follow-up period, referred that the odds ratio for the development of renal insufficiency (eGFR <60 mL/min per 1.73 m^2) increased dramatically when UA level exceeded 7.0 mg/dL in women and 8.0 mg/dL in men. The UA level between 7.0 and 8.9 mg/dL was associated with a nearly doubled risk for incident kidney disease and those with UA levels >9.0 mg/dL had a tripled risk (37). Another study that enrolled patients with nephrosclerosis suggested that the optimal UA cutoff value for predicting an eGFR decline by >50% from baseline or ESRD was 8.0 mg/dL (23). In our investigation, the reduction of eGFR was not observed in patients with UA >7.0 mg/dL but was significant if the cutoff value was set at 7.5 mg/dL or higher. This result served as important information for both physicians and patients in predicting the future risk of renal diseases. By initiating the evaluation earlier, we hope to delay the development of CKD in patients with hypertension.

The definition for hyperuricemia is gender-specific (10, 12). Therefore, whether there are different UA thresholds for predicting renal impairment in male and female is of our interest. One previous study suggested that the risk for incident kidney disease was associated with gender. The risk increased as UA level exceeded 6 to 7 mg/dL in women and 7 to 8 mg/dL in men (37). Our subgroup analysis seemed to provide gender-specific UA cutoff value as well, female as 8.0 mg/dL and male as 7.5 mg/dL, for minor renal event. However, none of the interaction tests was significant. This finding was consistent with one meta-analysis, which revealed no difference between men and women in UA level and CKD (38).

Despite numerous studies indicating the association between UA levels and renal diseases, data on the effects of uric acid-lowering agents on renal outcomes are limited and inconsistent. Three randomized, controlled trials, conducted in Hong Kong, Spain, and Iran, respectively, revealed that fewer patients in the allopurinol group endorsed renal function deterioration compared to the control group (39–41). However, several studies have shown different outcomes. The CKD-FIX Study (randomized Controlled trial of slowing of Kidney Disease progression From the Inhibition of Xanthine oxidase), enrolling

a total of 363 patients with stage 3 or 4 CKD, concluded that allopurinol did not appear to effectively alter the progression of renal insufficiency during a 2-year follow-up (42). One of the possible explanations for this opposite result is that the study did not include UA level–based criteria at enrollment. Therefore, some participants had normal UA levels, while others had elevated UA levels. On the other hand, when comparing different urate-lowering agents, febuxostat reduced UA more and earlier than allopurinol (43). However, there was no difference in the decline of renal function between the two groups during a 3-year period (44). Therefore, additional comprehensive trials involving a larger cohort of participants to determine the long-term efficacy of different urate-lowering agents, as well as to characterize sub-populations who would benefit from urate-lowering agents, would be essential.

Study Limitations

This study has several limitations that must be addressed. First, this was an observational study. There may have been a selection bias in patient enrollment. However, we tried our best to exclude participants with diabetes and other comorbidities to attenuate the impacts of other factors related to renal function deterioration. Second, the number of participants and major renal events was relatively small. Though only 11 participants experienced major renal events, there were 52 participants meet the criteria of minor renal events. The impacts of baseline UA levels on major and minor events were consistent, which increased the strengths of our study. Further studies with large sample size will be indicated. Third, our study was conducted only in Chinese patients with hypertension in Taiwan. Since UA levels may vary between different ethnic backgrounds (45, 46), our findings should be tested in hypertensive patients with different ethnic backgrounds in the future. Fourth, we did not investigate the impact of uric acid-lowering agents on renal function. To better clarify the relationship between UA levels and renal function, patients treated with urate-lowering agents were excluded from our study. However, whether hypertensive patients would benefit from early intervention for hyperuricemia is unknown. Therefore, further interventional trials should be conducted to determine the efficacy of urate-lowering agents for renal protection. Finally, although the hypertensive patients in our cohort were educated for dietary modification during the out-patient clinic follow-up, we did not have detail information about the dietary. Further studies with detail dietary information will be indicated.

CONCLUSION

A high serum UA level is a significant risk factor for the decline in renal function in Han Chinese hypertensive patients in Taiwan. Patients with a baseline UA level $\geq$7.5 mg/dL were associated with minor or major nephropathy. Our findings support the routine measurement of serum UA levels in hypertensive patients to identify those who are more susceptible to the development of nephropathy. However, further studies are needed to clarify whether early intervention with urate-lowering agents could prevent renal impairment in hypertensive patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Taipei Veterans General Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Y-HH, C-CH, and L-YL: conceptualization. C-CH: methodology and formal analysis, resources and data curation, writing—review and editing, project administration, and funding acquisition. Y-HH, L-YL, and J-WC: writing—original draft preparation. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by research grant V110C-058, V111C-086, and V111EA-014 from Taipei Veterans General Hospital, Taipei, Taiwan, R.O.C., and research grant MOST 108-2314-B-075-062-MY3 from the Ministry of Science and Technology, Taiwan, R.O.C.

REFERENCES

1. Ku E, Lee BJ, Wei J, Weir MR. Hypertension in CKD: core curriculum 2019. *Am J Kidney Dis.* (2019) 74:120–31. doi: 10.1053/j.ajkd.2018.12.044
2. Dionne JM, Jiang S, Ng DK, Flynn JT, Mitsnefes MM, Furth SL, et al. Mean arterial pressure and chronic kidney disease progression in the CKiD cohort. *Hypertension.* (2021) 78:65–73. doi: 10.1161/HYPERTENSIONAHA.120.16692
3. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS. Uric acid and incident kidney disease in the community. *J Am Soc Nephrol.* (2008) 19:1204–11. doi: 10.1681/ASN.2007101075
4. Iseki K, Ikemiya Y, Inoue T, Iseki C, Kinjo K, Takishita S. Significance of hyperuricemia as a risk factor for developing ESRD in a screened cohort. *Am J Kidney Dis.* (2004) 44:642–50. doi: 10.1016/S0272-6386(04)00934-5
5. Chang YH, Lei CC, Lin KC, Chang DM, Hsieh CH, Lee YJ. Serum uric acid level as an indicator for CKD regression and progression in patients with type 2 diabetes mellitus-a 4.6-year cohort study. *Diabetes Metab Res Rev.* (2016) 32:557–64. doi: 10.1002/dmrr.2768
6. Ficociello LH, Rosolowsky ET, Niewczas MA, Maselli NJ, Weinberg JM, Aschengrau A, et al. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: results of a 6-year follow-up. *Diabetes Care.* (2010) 33:1337–43. doi: 10.2337/dc10-0227
7. Zoppini G, Targher G, Chonchol M, Ortalda V, Abaterusso C, Pichiri I, et al. Serum uric acid levels and incident chronic kidney disease in patients

with type 2 diabetes and preserved kidney function. *Diabetes Care.* (2012) 35:99–104. doi: 10.2337/dc11-1346
8. Russo E, Viazzi F, Pontremoli R, Barbagallo CM, Bombelli M, Casiglia E, et al. Association of uric acid with kidney function and albuminuria: the Uric Acid Right for heArt Health (URRAH) Project. *J Nephrol.* (2021). doi: 10.1007/s40620-021-00985-4. [Epub ahead of print].
9. Watanabe K, Nakayama M, Yamamoto T, Yamada G, Sato H, Miyazaki M, et al. Different clinical impact of hyperuricemia according to etiologies of chronic kidney disease: Gonryo study. *PLoS ONE.* (2021) 16:e0249240. doi: 10.1371/journal.pone.0249240
10. Bardin T, Richette P. Definition of hyperuricemia and gouty conditions. *Curr Opin Rheumatol.* (2014) 26:186–91. doi: 10.1097/BOR.0000000000000028
11. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yü TF. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum.* (1977) 20:895–900. doi: 10.1002/art.1780200320
12. Desideri G, Castaldo G, Lombardi A, Mussap M, Testa A, Pontremoli R, et al. Is it time to revise the normal range of serum uric acid levels? *Eur Rev Med Pharmacol Sci.* (2014) 18:1295–306.
13. FitzGerald JD, Dalbeth N, Mikuls T, Brignardello-Petersen R, Guyatt G, Abeles AM, et al. 2020 American College of Rheumatology Guideline for the Management of Gout. *Arthritis Care Res.* (2020) 72:744–60. doi: 10.1002/acr.24375
14. Richette P, Doherty M, Pascual E, Barskova V, Becce F, Castañeda-Sanabria J, et al. 2016 updated EULAR evidence-based recommendations for the management of gout. *Ann Rheum Dis.* (2017) 76:29–42. doi: 10.1136/annrheumdis-2016-209707
15. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
16. Tsai WC, Wu HY, Peng YS, Yang JY, Chen HY, Chiu YL, et al. Association of intensive blood pressure control and kidney disease progression in nondiabetic patients with chronic kidney disease: a systematic review and meta-analysis. *JAMA Intern Med.* (2017) 177:792–9. doi: 10.1001/jamainternmed.2017.0197
17. Kao TW, Huang CC, Chen JW. Optimal blood pressure for the prevention of hypertensive nephropathy in nondiabetic hypertensive patients in Taiwan. *J Clin Hypertens.* (2020) 22:1425–33. doi: 10.1111/jch.13956
18. McClellan WM, Flanders WD. Risk factors for progressive chronic kidney disease. *J Am Soc Nephrol.* (2003) 14(Suppl. 2):S65–70. doi: 10.1097/01.ASN.0000070147.10399.9E
19. Kuo HW, Tsai SS, Tiao MM, Yang CY. Epidemiological features of CKD in Taiwan. *Am J Kidney Dis.* (2007) 49:46–55. doi: 10.1053/j.ajkd.2006.10.007
20. Moriyama T, Itabashi M, Takei T, Kataoka H, Sato M, Shimizu A, et al. High uric acid level is a risk factor for progression of IgA nephropathy with chronic kidney disease stage G3a. *J Nephrol.* (2015) 28:451–6. doi: 10.1007/s40620-014-0154-0
21. Ohno I, Hosoya T, Gomi H, Ichida K, Okabe H, Hikita M. Serum uric acid and renal prognosis in patients with IgA nephropathy. *Nephron.* (2001) 87:333–9. doi: 10.1159/000045939
22. Syrjänen J, Mustonen J, Pasternack A. Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. *Nephrol Dial Transplant.* (2000) 15:34–42. doi: 10.1093/ndt/15.1.34
23. Momoki K, Kataoka H, Moriyama T, Mochizuki T, Nitta K. Hyperuricemia as a predictive marker for progression of nephrosclerosis: clinical assessment of prognostic factors in biopsy-proven arterial/arteriolar nephrosclerosis. *j Atheroscler Thromb.* (2017) 24:630–42. doi: 10.5551/jat.37523
24. Huang Y, Li YL, Huang H, Wang L, Yuan WM, Li J. Effects of hyperuricemia on renal function of renal transplant recipients: a systematic review and meta-analysis of cohort studies. *PLoS ONE.* (2012) 7:e39457. doi: 10.1371/journal.pone.0039457
25. Weng SC, Shu KH, Wu MJ, Cheng CH, Chen CH, Yu TM, et al. Hyperuricemia predicts kidney disease progression after acute allograft dysfunction. *Transplant Proc.* (2014) 46:499–504. doi: 10.1016/j.transproceed.2013.09.040
26. Moe OW. Posing the question again: does chronic uric acid nephropathy exist? *J Am Soc Nephrol.* (2010) 21:395–7. doi: 10.1681/ASN.2008101115
27. Mulay SR, Shi C, Ma X, Anders HJ. Novel insights into crystal-induced kidney injury. *Kidney Dis.* (2018) 4:49–57. doi: 10.1159/000487671
28. Johnson RJ, Kivlighn SD, Kim YG, Suga S, Fogo AB. Reappraisal of the pathogenesis and consequences of hyperuricemia in hypertension, cardiovascular disease, and renal disease. *Am J Kidney Dis.* (1999) 33:225–34. doi: 10.1016/S0272-6386(99)70295-7
29. Lusco MA, Fogo AB, Najafian B, Alpers CE. AJKD atlas of renal pathology: gouty nephropathy. *Am J Kidney Dis.* (2017) 69:e5–6. doi: 10.1053/j.ajkd.2016.11.006
30. Jalal DI, Maahs DM, Hovind P, Nakagawa T. Uric acid as a mediator of diabetic nephropathy. *Semin Nephrol.* (2011) 31:459–65. doi: 10.1016/j.semnephrol.2011.08.011
31. Hovind P, Rossing P, Tarnow L, Johnson RJ, Parving HH. Serum uric acid as a predictor for development of diabetic nephropathy in type 1 diabetes: an inception cohort study. *Diabetes.* (2009) 58:1668–71. doi: 10.2337/db09-0014
32. Hovind P, Rossing P, Johnson RJ, Parving HH. Serum uric acid as a new player in the development of diabetic nephropathy. *J Ren Nutr.* (2011) 21:124–7. doi: 10.1053/j.jrn.2010.10.024
33. Ejerblad E, Fored CM, Lindblad P, Fryzek J, Dickman PW, Elinder CG, et al. Association between smoking and chronic renal failure in a nationwide population-based case-control study. *J Am Soc Nephrol.* (2004) 15:2178–85. doi: 10.1097/01.ASN.0000135048.35659.10
34. Locatelli F, Pozzoni P, Del Vecchio L. Renal manifestations in the metabolic syndrome. *J Am Soc Nephrol.* (2006) 17(Suppl. 2):S81–5. doi: 10.1681/ASN.2005121332
35. Davis TM, Ting R, Best JD, Donoghoe MW, Drury PL, Sullivan DR, et al. Effects of fenofibrate on renal function in patients with type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study. *Diabetologia.* (2011) 54:280–90. doi: 10.1007/s00125-010-1951-1
36. Sandhu S, Wiebe N, Fried LF, Tonelli M. Statins for improving renal outcomes: a meta-analysis. *J Am Soc Nephrol.* (2006) 17:2006–16. doi: 10.1681/ASN.2006010012
37. Obermayr RP, Temml C, Gutjahr G, Knechtelsdorfer M, Oberbauer R, Klauser-Braun R. Elevated uric acid increases the risk for kidney disease. *J Am Soc Nephrol.* (2008) 19:2407–13. doi: 10.1681/ASN.2008010080
38. Zhu P, Liu Y, Han L, Xu G, Ran JM. Serum uric acid is associated with incident chronic kidney disease in middle-aged populations: a meta-analysis of 15 cohort studies. *PLoS ONE.* (2014) 9:e100801. doi: 10.1371/journal.pone.0100801
39. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. *Am J Kidney Dis.* (2006) 47:51–9. doi: 10.1053/j.ajkd.2005.10.006
40. Goicoechea M, de Vinuesa SG, Verdalles U, Ruiz-Caro C, Ampuero J, Rincón A, et al. Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. *Clin J Am Soc Nephrol.* (2010) 5:1388–93. doi: 10.2215/CJN.01580210
41. Momeni A, Shahidi S, Seirafian S, Taheri S, Kheiri S. Effect of allopurinol in decreasing proteinuria in type 2 diabetic patients. *Iran J Kidney Dis.* (2010) 4:128–32.
42. Badve SV, Pascoe EM, Tiku A, Boudville N, Brown FG, Cass A, et al. Effects of allopurinol on the progression of chronic kidney disease. *N Engl J Med.* (2020) 382:2504–13. doi: 10.1056/NEJMoa1915833
43. Sezai A, Soma M, Nakata K, Osaka S, Ishii Y, Yaoita H, et al. Comparison of febuxostat and allopurinol for hyperuricemia in cardiac surgery patients with chronic kidney disease (NU-FLASH trial for CKD). *J Cardiol.* (2015) 66:298–303. doi: 10.1016/j.jjcc.2014.12.017
44. Kojima S, Matsui K, Hiramitsu S, Hisatome I, Waki M, Uchiyama K, et al. Febuxostat for Cerebral and CaRdiorenovascular Events PrEvEntion StuDy. *Eur Heart J.* (2019) 40:1778–86. doi: 10.1093/eurheartj/ehz119
45. Singh JA. Racial and gender disparities among patients with gout. *Curr Rheumatol Rep.* (2013) 15:307. doi: 10.1007/s11926-012-0307-x

46. Butler F, Alghubayshi A, Roman Y. The epidemiology and genetics of hyperuricemia and gout across major racial groups: a literature review and population genetics secondary database analysis. *J Pers Med.* (2021) 11:231. doi: 10.3390/jpm11030231

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

MINI REVIEW
published: 02 February 2022
doi: 10.3389/fmed.2021.784435

Assessing Renal Function for Kidney Donation. How Low Is Too Low?

Gustavo Laham, Juan Pablo Ponti and Gervasio Soler Pujol*

Internal Medicine Department, Nephrology Section, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Buenos Aires, Argentina

Kidney transplantation (KT) is the treatment of choice for patients with end-stage kidney disease (ESKD) with decreased morbi-mortality, improved life quality, and reduced cost. However, the shortage of organs from deceased donors led to an increase in KT from living donors. Some stipulate that living donors have a higher risk of ESKD after donation compared with healthy non-donors. The reason for this is not clear. It is possible that ESKD is due to the nephrectomy-related reduction in glomerular filtration rate (GFR), followed by an age-related decline that may be more rapid in related donors. It is essential to assess donors properly to avoid rejecting suitable ones and not accepting those with a higher risk of ESKD. GFR is a central aspect of the evaluation of potential donors since there is an association between low GFR and ESKD. The methods for assessing GFR are in continuous debate, and the kidney function thresholds for accepting a donor may vary according to the guidelines. While direct measurements of GFR (mGFR) provide the most accurate evaluation of kidney function, guidelines do not systematically use this measurement as a reference. Also, some studies have shown that the GFR decreases with age and may vary with gender and race, therefore, the lower limit of GFR in patients eligible to donate may vary based on these demographic factors. Finally, it is known that CrCl overestimates mGFR while eGFR underestimates it, therefore, another way to have a reliable GFR could be the combination of two measurement methods.

Keywords: end stage kidney disease (ESKD), kidney transplant, glomerular filtration rate, estimated GFR, measure GFR

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Carlos Arias-Cabrales, Parc de Salut Mar, Spain
Ryoichi Imamura, Osaka University, Japan

****Correspondence:***
Gustavo Laham
guslaham@yahoo.com.ar

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *27 September 2021*
Accepted: *09 December 2021*
Published: *02 February 2022*

Citation:
Laham G, Ponti JP and Soler Pujol G (2022) Assessing Renal Function for Kidney Donation. How Low Is Too Low? Front. Med. 8:784435. doi: 10.3389/fmed.2021.784435

INTRODUCTION

The number of patients with end-stage kidney disease (ESKD) requiring dialysis is increasing around the world (1). This has been mainly attributed to the rising prevalence of diabetes mellitus (DM) and hypertension (HTN) in an elder population that reaches this stage (2). Not surprisingly this is associated with increased morbidity, mortality, healthcare costs, and reduced quality of life (3–7). Our country, Argentina, is not an exception; in 2019, there were 30,432 prevalent patients on dialysis with an incidence and a prevalence of 164 and 677 patients per million inhabitants, respectively (8). The annual mortality rate for patients with chronic kidney disease (CKD) stage 5D is 17.3%, being 14.7% for peritoneal dialysis, and 17.7 % for patients on hemodialysis (8).

Today, renal transplantation (RT) is the best treatment option for patients reaching ESKD because it is associated with better patient survival, quality of life, and lower costs compared with patients remaining on dialysis (9). Unfortunately, as time goes by the increasing number of patients reaching ESKD combined with organ shortage makes waiting time for deceased donors RT longer (8). In Argentina, the number of patients on dialysis has increased while the number of RT has remained stable over the years at a rate of 19–21 RT per million inhabitants. The rate increased

in 2018 and 2019 to 24.6 and 29.5 per million inhabitants, respectively, at the expenses of deceased donors. However, the percentage of RT with living donors has not increased for the past 10 years, being approximately 20% of all kidney transplants (8).

One strategy to cope with the increasing number of patients on the waiting list is to increase the living kidney donor (LKD) pool with more flexible donor acceptance criteria (10).

One of the major concerns about LKD is whether they have the same mortality and life expectancy compared with a similar age, sex, and comorbidity matched population. In a study by Sergev et al. (11), the risk of death in the first 90 days following live donor nephrectomy was 3.1 per 10,000 donors (95% *CI*, 2.0–4.6). Mortality was higher in men, African Americans, and with a history of HTN. This mortality rate seems low compared with that reported in the literature of laparoscopic cholecystectomy 15/10,000 (12) or nephrectomy of non-donor 260/10,000 surgeries (13). Others studies addressed the long-term risk of death and found that the life expectancy of kidney donors appears to be similar to that of non-donors or perhaps even longer (14, 15). Additionally, Ibrahim et al. (15) compared 3,698 kidney donors' survival who donated kidneys during the period from 1963 through 2007 with the general population from the National Center for Health Statistics. The survival of kidney donors was similar to that of controls who were matched for age, sex, and race or ethnic group. On the other hand, Geir Mjoen et al. (16) analyzed 1,901 subjects who donated kidneys from 1963 to 2007 with a median follow-up of 15.1 years. A control group of 32,621 potentially suitable kidney donors was selected, with a follow-up of 24.9 years. Hazard ratio (*HR*) for all-cause death was significantly increased to 1.30 (95% *CI* 1.11–1.52) for donors compared with controls. One of the reasons that could explain this finding was that the control groups were all from the same county with a higher life expectancy compared with Norway where the donors came from. More recently Shiromani Janki et al. (17) compared 761 LKDs, who visited the outpatient clinic and their propensity score was matched with 1,522 non-donors from population-based cohort studies. The study showed no significant differences between donors and non-donors in overall and cardiovascular mortality.

Another concern in LKD is about the risk of progression to ESKD. Nevertheless, several studies have shown no differences between LKD and matched controls in this respect (16, 18, 19). The study by Ibrahim et al. (15) suggests that there is no excessive risk of ESKD to donors and confirms the view that factors associated with reduced GFR in donors are similar to those that have been observed in the general population. The estimated incidence of ESKD in donors would appear to be 180 per million persons per year, as compared with the overall adjusted incidence rate of 268 per million persons per year in the white population of the United States (15). Between 1996 and 2015, there has been an increase in the number of LKD reaching the United States kidney waiting list with a total of 441 patients (20). In 2014, Muzaale et al. (21) compared 96,217 LKD from the Organ Procurement and Transplantation Network (OPTN) registry (1994–2007) with 20,024 participants of the NHANES III study showing a low incidence of ESKD, 15 years post-donation (estimated risk of 30.8 of 10,000 donors over 15 years). This represented an 8-fold increased incidence of ESKD in kidney donors compared with healthy individuals in the United States who had not donated. This difference was seen in both black and white subjects, with an estimated risk of 74.7 per 10,000 black donors vs. 23.9 per 10,000 black non-donors ($p < 0.001$) and estimated risk of 22.7 per 10,000 white donors vs. 0.0 per white non-donors ($p < 0.001$).

Even though African American (AA) had a higher risk of ESKD in the general population compared with white individuals, the absolute risk of ESKD in black living donors was also low. Finally, the estimated lifetime risk of ESKD was 90 per 10,000 donors, 326 per 10,000 unscreened non-donors (general population), and 14 per 10,000 healthy non-donors (21). The question is whether LKDs develop ESKD as a consequence of new-onset disease that can affect the remaining kidney or is it due to a constant fall in the glomerular filtration rate (GFR) that can occur with aging. Recently a study published by Matas et al. (22) over 40,130 LKDs from 1963 to 2015, 39 developed ESKD, (mean age at ESKD, 62.4 years; mean interval between donation and ESKD, 27.1 $\pm$ 9.8 years). Donors who developed ESKD were more likely to be men, as well as smokers, younger at the time of donation, and to have donated to a first-degree relative. Of donors with a known cause of ESKD ($n = 25$), 48% was due to diabetes mellitus (DM) and/or hypertension (HTN), and only two were from a disease that would have affected one kidney (cancer). Therefore, knowing the risk factors for ESKD in LKDs could help improve their selection as well as their medical care after donation. In fact, today LKD tends to be older and have more comorbidities which call for a thorough evaluation before donation (23). O'Keeffe et al. (24) published a systematic review and meta-analysis of 52 studies, including 118,426 LKDs and 117,656 non-donors. They did not find evidence suggesting a higher risk for cardiovascular disease, type 2 diabetes, or adverse psychosocial health outcomes in LKDs than in non-donor populations. On the other hand, kidney donors had higher diastolic blood pressure and a higher risk for preeclampsia in female donors. Although living kidney donation is associated with higher relative risks (RRs) for preeclampsia, the absolute risk for this outcome remains low, compared with non-donor populations. Similar results were obtained by Janki et al. (17), but there was a lower risk of new-onset HTN compared with the meta-analysis (24). Kasiske et al. (25), in a 3-year study, compared 182 donors and 173 non-donors. They found that donors at 3 years follow-up had significantly higher levels of uric acid, phosphorus, and parathyroid hormone (PTH). This could have been related to a decrease in kidney mass in apparently healthy individuals. Whether this has any implications for the bone health of donor is not yet known. In **Table 1**, we summarized the most relevant studies concerning mortality and progression to ESKD.

There is little consensus about what is considered an appropriate GFR threshold for donation and formulation of guidelines is hampered by the heterogeneity of practice in how GFR is measured, uncertainty on the accuracy of GFR estimating equations, and bias and imprecision of standard methods of GFR measurement. The aim of this review is to update the different methods of GFR assessment in live kidney donors, their strengths, and limitations. In addition, we will go through the

TABLE 1 | Summary of the most relevant studies related to post-donation medical outcomes.

	Study design	Outcome: Comparison of LKD to Controls
Mortality		
Sergev et al. (11)	80,347 LKD vs. Controls matched from NHANES III by age, gender, race, BMI, smoking history, and SBP, after exclusions for baseline comorbidity	Risk of death in the first 90 days after live donor nephrectomy (3.1/10,000) Long-term LKD mortality not higher vs. matched healthy
Ibrahim et al. (15)	3,698 LKD survival vs. NHANES controls. 1:1 ratio, matched by: age, race and BMI	No differences in mortality between groups
Gier Mjoen et al. (16)	1,901 LKD vs. 32,621 subjects between the ages of 20 and 70, general Norwegian population, with no contraindication to donation of a kidney Matching on age, sex, SBP, BMI and smoking	Risk for LKD: HR for all-cause death: 1.30 (1.11–1.52) and cardiovascular death: 1.40 (1.03–1.91)
Shiromani Janki et al. (17)	761 LKD vs. 1,522 non donors	No differences in overall mortality (OR 0.06, 95% CI 0.05; 0.08) for LKD
Progression to ESKD		
Muzaale et al. (21)	96,217 LKD vs. 20,024 patients from the NHANES III study. Excluding those with contraindication to kidney donation: $n =$ 9,364 subjects matching on age, sex, race, BMI, smoking and SBP	Incidence of ESKD 30.8/10,000 donors over 15 years vs. 3.9/10,000 in healthy non-donors
Gier Mjoen et al. (16)	1,901 LKD vs. 32,621 subjects between the ages of 20 and 70, general Norwegian population, with no contraindication to donation of a kidney Matching on age, sex, SBP, BMI and smoking	HR for ESKD in LKD: 11.38 (4.37–29.63, $P < 0.001$)
Ibrahim et al. (15)	3,698 LKD survival vs. NHANES controls. 1:1 ratio, matched by: age, race and BMI	No difference in incidence of ESKD: 180 per million persons per year in LKD vs. 268 per million persons per year in controls.
O'Keeffe et al. (24)	Meta-analysis: 52 studies, 118,426 LKD vs. 117,656 controls	LKD: higher diastolic blood pressure, lower estimated glomerular filtration rates. Risk for ESKD (RR, 8.83 [CI, 1.02 to 20.93]), but low absolute risk for ESKD (incidence rate, 0.5 event [CI, 0.1 to 4.9 events] per 1000 person-years)

LKD, living kidney donor; NHANES III, National Health and Nutrition Examination Study; SBP, Systolic blood pressure; ESKD, end stage kidney disease; BMI, body mass index.

current guidelines of GFR thresholds available in the literature, and finally, we will analyze the impact of pre-donation GFR on post-donation outcomes.

METHODS

A non-language restricted search was performed until August 30, 2021 in PubMed, SciELO, Trip Database, Google Scholar, and MEDES y MEDLINE, using the following MeSH terms and key words, "living kidney donors," "chronic kidney disease," "donor nephrectomy," "glomerular filtration rate," "estimate glomerular filtration rate," "glomerular filtration rate," "glomerular filtration rate measurements," "cystatin C," "CKD-EPI," "MDRD," "creatinine clearance," "inulin," "iothalamate," "diethylenetriaminepentaacetic acid," "iohexol," and "ethylenediamine tetra-acetic acid." Bibliographies of relevant articles and reviews were manually screened to identify additional studies.

GFR in Normal Subjects

A precise assessment of pre-donation GFR and its follow-up is essential to identify donors at risk to initiate prevention strategies.

Glomerular filtration rate has been established for decades by measuring the 24-urine creatinine excretion (26). One of the problems with this method is that in healthy subjects, it overestimates GFR by up to 10% due to tubular excretion of creatinine (27). The others relate to the possible imprecision and bothersome 24 h urine collection. Therefore, other methods to measure GFR (mGFR) have taken place. The exogenous tracers are considered the gold standard (28), such as inulin, technetium-99mTc-diethylenetriaminepentaacetic acid (99mTc-DTPA) (29), 51Cr-ethylenediaminetetraacetic acid (EDTA) (30), 125I-iothalamate (31), and non-reactive Iohexol. Nevertheless, they are expensive, their use is complex, and may have side effects. For this reason, GFR is frequently estimated by equations (eGFR) which incorporate endogenous markers, as well as demographic and anthropometric parameters (27). Among those using creatinine, the chronic kidney disease epidemiology (CKD-EPI) (32) is the most widely used. Other equations include cystatin C (CysC) (33), CysC +Cr (34), beta-trace protein (BTP), and beta2 microglobulin (B2M) (35).

Most of the studies for mGFR and eGFR have been designed and validated in renal patients with chronic kidney disease (CKD) therefore their assessment of GFR in normal individuals, such as LKD, is not precise.

GFR Measurement

Inulin is a fructose polysaccharide found in the roots of a variety of plants. It has a molecular weight of approximately 5,200 Da. It is neither metabolized nor reabsorbed or secreted by the renal tubules, therefore, it can be quantitatively recovered in the urine after intravenous administration. As described by Homer Smith in 1935 (36), Inulin clearance is constant and

independent of its plasmatic concentration. Experimental studies have shown similar concentrations in the Bowman's space and plasma, 99.3% of Inulin injected in the proximal tubule is present in the distal tubule (37). Inulin clearance continues to be the gold standard for the measurement of GFR. Nevertheless, issues concerning its cost, the complexity of the procedure, and scarce availability turns its clinical use generally infeasible. Many procedures to measure mGFR (38) have been developed, all of them are more imprecise than the inulin clearance which is already not 100% precise as it has a coefficient of variation of approximately 7% on repeated measurements in the same subjects (39). One study addressed this issue by comparing the accuracy of mGFR measured by other exogenous tracers to that of inulin. They found that both plasmatic or urinary clearance of 125I-iothalamate, EDTA, or Iohexol, and only the urinary clearance of Tc99-DTPA is sufficiently accurate to measure GFR (40).

Iothalamate is currently used in the United States. It is an ionic contrast tracer with a molecular weight of approximately 637 Da, very little protein-bound (41). It has a good correlation with inulin clearance but as it is secreted and reabsorbed by renal tubules, it has been shown to overestimate GFR (42, 43).

Ethylenediaminetetraacetic acid is available in Europe. Its cost is high as it requires all the precautions for storage, administration, and disposal of radioactive substances. It may have tubular reabsorption as it tends to underestimate GFR compared with inulin (44). Another compound, DTPA is a chelating agent used in the United States and Canada. It produces radiochemical debris while labeling with Tc99. It is protein-bound in about 4–10% increasing its permanence in the circulation which tends to underestimate GFR (45).

Iohexol is a non-ionic, non-radioactive, low-osmolality contrast dye developed in the 1980s (46). It has a molecular weight of 821.1 Da. Its protein-bound is scarce (47). It is cheap, safe, and widely used. Methods taking series of blood samples after tracer injection are popular although not validated for subjects with normal renal function. Only one study measured Iohexol plasma clearance in 20 patients without kidney disease (48). The mGFR obtained after 5 samples (5M) (150/180/200/220/240 min) or 4 samples (4M) (180/200/220/240 min) was compared with that of inulin. The mGFR with the sample taken at 150 min in M5 was the closest to inulin's but still underestimated GFR by 13 ml/min/1.73 m^2. Authors postulated that if an earlier sample could be more accurate in normal subjects.

GFR Estimation

Due to the limitations described for mGFR, equations have been developed to estimate GFR (eGFR). As we mentioned they are based on endogenous substances. Creatinine (Cr) is the most widely used. Cr is a 113 Da amino acid generated in the muscle from ingested food. It spreads in total body water, it is filtered by the glomerulus, secreted by the tubules, and excreted in the urine. We will focus on two equations, modification of diet in renal disease (MDRD) and CKD-EPI. None of them were developed in healthy subjects (49). MDRD was developed in 1999 (50) with creatinine standardization by the isotope dilution mass spectrometry in 2006. Compared with Cockroft-Gault, MDRD is more accurate (51). The most important limitation of this formula is that it underestimates GFR with values over 60 ml/min/1.73 m^2. Different from MDRD, CKD-EPI was validated in a cohort including not only patients with CKD but also individuals with normal renal function which provides a better correlation with normal subjects (52).

The performance of these equations in LKD has been explored. In one study (53), the accuracy of MDRD and CKD-EPI for different clinical situations including LKD ($n = 583$) was evaluated. For the detection of an mGFR < 60 ml/min/1.73 m^2, CKD-EPI had a sensitivity of 50% and specificity of 98%, while MDRD had 70 and 94%, respectively. For the detection of an mGFR < 80 ml/min/1.73 m^2, CKD-EPI had a sensitivity of 71% and specificity of 76% while MDRD had 89 and 48%, respectively.

Another study (54) evaluating LKD found that the probability of having an mGFR < 80 ml/min/1.73 m^2 with the eGFR < 80 ml/min/1.73 m^2 was 14%, in other words, 86% of discharged donors by eGFR would have been donors by mGFR. Therefore, the use of the eGFR with creatinine for LKD selection is flawed by the risk of rejecting candidates with a falsely low eGFR and accepting others with a falsely high eGFR. Another endogenous substance used to estimate GFR is Cystatin C (CysC), a protein that inhibits cysteine and is secreted by the majority of cells. CysC is freely filtered by the glomerulus, almost fully reabsorbed, and metabolized by tubular epithelial cells, it is not secreted and urinary excretion is negligible. Blood levels are less affected than creatinine by body mass, diet, age, or sex (55). Nevertheless, they can be affected by hyperthyroidism, high-dose steroids, and cardiovascular disease (56).

There are several equations available that incorporate CysC either alone or in combination with creatinine to estimate GFR. In patients with CKD, it has been demonstrated that eGFR by CKD-EPI with CysC+Cr has higher precision than CKD-EPI only with Cr (34). One study (57) performed in 147 potential LKD found that the combined equation underestimated mGFR less than the only Cr-based equation ($-2{,}7$ vs. -11.6 ml/min/1.73 m^2). The greater difference was observed with mGFR between 89 and 60 ml/min/1.73 m^2 where CycC+Cr had -4.3 ml/min/1.73 m^2 vs. -15 ml/min/1.73 m^2 for the only Cr based equation.

Nevertheless, there are circumstances where the eGFR + CysC is decreased compared with eGFR + Cr or mGFR. The so-called "shrunken pore syndrome" has been defined when eGFR + CysC is less than 60% of the eGFR + Cr (58). In 2015, it was described in pregnant women in their third trimester (59). It has also been associated with increased mortality after cardiac surgery (60) and decreased left ventricular systolic function (61). Its relevance to LKD has not been yet evaluated.

A Spanish group (62) compared mGFR by iohexol with that of 51 different equations based on Cr, CysC or both, in the selection of 103 LKD. The threshold for donation was established at mGFR > 80 ml/min for those >35 years old or 90 ml/min for those <35 years old. In total, 93 subjects (90.3%) had mGFR over the threshold and 10 (9.7%) below the threshold. Many of those not selected by mGFR were over the threshold by eGFR and would have been selected. All of those excluded were women. In subjects

selected by mGFR, 32 were below the threshold by eGFR that would have left them out for donation.

Guidelines for GFR Evaluation Before LKD

Several guidelines are addressing this matter. They use different methods to evaluate GFR and different GFR thresholds. We will summarize the ones we considered the most relevant (**Table 2**).

Kidney Disease: Improving Global Outcomes-KDIGO 2017

The initial recommended test is CKD-EPI + Cr, and then confirmatory testing, as needed. Depending on availability, mGFR, with exogenous or endogenous markers, eGFR combining CysC + Cr or repeating an eGFR Cr can be used (63).

Another tool suggested by the guideline is a web-based calculator to estimate the probability of having an mGFR below 60, 70, 80, and 90 ml/min/1.73 m^2 (http://ckdepi.org/equations/donor-candidate-GFR-calculator/). It is divided into two steps. First, it calculates the pre-test probability of having an mGFR below 60, 70, 80, and 90 ml/min/1.73 m^2 based on gender, age, and ethnicity. It then performs a post-test taking into account creatinine measurements with or without cystatin, using data obtained from the CKD-EPI cohort and eGFR/mGFR concordance. For example, a 25-year-old white male with a plasma creatinine of 1 mg/dl (eGFR CKD-EPI 104 ml/min/1.73 m^2) has a post-test probability of having an mGFR of less than 90 ml/min/1.73 m^2 of 3%.

To accept a candidate routinely, they suggest an eGFR > 90 ml/min/1.73 m^2 and < 60 ml/min/1.73 m^2 to exclude the participant. For values in the middle decisions should be individualized and other risk factors should be considered. This recommendation was based on a meta-analysis with almost 5 million healthy subjects where they found that for an eGFR > 90 ml/min/1.73 m^2, the life-long risk of developing CKD was approximately 1% of any age and race. For subjects aged 60 years or older with an eGFR between 60 and 89 ml/min/1.73 m^2, the risk is less than 1% (64).

British Transplantation Society-BTS 2018

Similar to KDIGO, they recommend CKD-EPI + Cr as the initial test. This will identify and rule out potential donors with CKD (eGFR < 45 ml/min/1.73 m^2). For the rest, GFR should be confirmed by mGFR with inulin clearance, 51Cr-EDTA, 125I-iothalamate, or iohexol (65).

Whenever there is a disparity between the two kidneys greater than 10%, they recommend performing a differential mGFR for each kidney with a combination of 51Cr-EDTA and 99mTc-DMSA. If eligible, the less functioning kidney should be donated.

Glomerular filtration rate thresholds for donation should be adapted for age and sex. A study performed in more than 1,800 potential LKDs found that until age of 40 years, GFR remained stable, thereafter it decreased 6.6 ml/min/1.73 m^2 per decade in men and 7.7 ml/min/1.73 m^2 in women (66). In patients older than 35 years, a GFR >80 ml/min/1.73 m^2 seems safe for donation. In those younger than 30 years, a more conservative approach is warranted as there is evidence of a greater risk of CKD in this population with GFR < 90 ml/min/1.73 m^2 (64).

TABLE 2 | Guidelines for glomerular filtration rate (GFR) evaluation before living kidney donation.

	KDIGO	BTS	CTS	OPTN
Initial evaluation	CKD-EPI+Cr	CKD-EPI+Cr	eGFR Cr (CKD-EPI/Cockcroft-Gault)	mGFR or CrCl
Confirmation	Depending on availability: -mGFR (exogenous or endogenous markers) -eGFR cycs+cr -repeat eGFR Cr	mGFR (inulin, 51Cr-EDTA, 125I-iothalamate or iohexol)	Two separate creatinine clearance or one mGFR (DTPA, EDTA, iohexol or iothalamate)	—
Threshold GFR (ml/min/1,73 m2)	Accept: >90 Exclude: <60 Individualize decisions: 89–60	Accept: Male: 20–29yrs: >90 30–55yrs: >80 60yrs: >76 65yrs: >71 70yrs: >67 75yrs: >63 80yrs: >58 Female: 20–29yrs: >90 30–50yrs: >80 55yrs: >75 60yrs: >70 65yrs: >64 70yrs: >59 75yrs: >54 80yrs: >49	Accept: 18–30yrs: ≥90 31–40yrs: ≥85 41–65yrs: ≥80 >65yrs: ≥75	Accept: CrCl > 80 or predicted GFR at 80yrs: > 40
Others	Web-based calculator to estimate the probability of having a mGFR below 60, 70, 80 and 90 mL/min/1.73 m2	Size difference > 10%: mGFR 51Cr-EDTA + 99mTc-DTPA. Donate the least functioning	Size difference > 1 cm: mGFR 99mTc-DTPA. If differential GFR is more than 5%: Donate the least functioning	—

Canadian Transplant Society-CTS 2015

The recommendations include 2 creatinine determinations along with CKD-EPI or Cockcroft-Gault eGFR. In addition, 2 separate creatinine clearance measurements or one mGFR by DTPA, EDTA, iohexol, or iothalamate (67).

Additionally, it is suggested that asymmetric kidneys (>1 cm) should be evaluated by 99mTc-DTPA mGFR. If differential GFR is more than 5%, the donor should be left with the best functioning kidney.

Glomerular filtration rate thresholds are dependent on age. Potential donors between 18 and 30 years old should have a GFR $\geq$ 90 ml/min/1.73 m^2 to be accepted, a GFR $\geq$ 85 ml/min/1.73 m^2 for donors between 31 and 40 years old, a GFR $\geq$ 80 ml/min/1.73 m^2 for those 41 to 65 years old, and a GFR $\geq$ 75 ml/min/1.73 m^2 for those >65 years old.

Organ Procurement and Transplantation Network-OPTN

In their guidelines, they propose mGFR using exogenous filtration markers or creatinine clearance.

They suggest that those with a creatinine clearance < 80 ml/min/1.73 m^2 (68), or a predicted GFR at age 80 years is < 40 ml/min/1.73 m^2 (69), should be excluded for dotation (70).

Which Are the Recommended Methods for the Assessment of Renal Function for the Selection of Kidney Donors

In nephrology, there are few situations where an accurate assessment of GFR is essential, kidney donation is one of them (71), but guidelines do not specify which method should be used (72). In addition, the GFR threshold for kidney donation matters (73). A study carried out in 2007 found that 90% of living kidney transplant programs in the United States used 24-h CrCl (74). This is not an exception in our country. A survey conducted in 28 transplant centers in Argentina showed that 78.5% used CrCl for the assessment of donor's kidney function, while others used eGFR or iothalamate Cl. To accept a kidney donor, a CrCl > 80 ml/min/1.73 m^2 was required by 71.4% of the physicians while 21.4% required CrCl > 90 ml/min/1.73 m^2 (Maldonado et al., SLAH congress). It is well known that CrCl overestimates GFR and can lead to the approval of donors with a lower GFR than the optimal adjusted for age and sex.

Many transplant programs around the world use other methods to measure kidney function, such as eGFR or mGFR. The availability of mGFR varies, especially in developing countries where health resources are limited and where living kidney donation can be the only source of kidney transplants (75). On the other hand, the use of eGFR in the evaluation and selection of LKDs is controversial, in part due to concerns about its accuracy and correlation with mGFR to accept or reject donors (76). An alternative approach is to adapt eGFR thresholds to the best locally available technique. Two recently published studies offer a web-based application using eGFR to compute the probability that the measured GFR of a donor candidate is higher than the threshold previously defined at 80 ml/min/1.73 m^2 (77, 78). These studies indicate that it is possible to define different eGFR thresholds for mGFR limits coping with a certain degree of uncertainty as to whether the mGFR threshold is reached. In agreement with these findings, the KDIGO Clinical Practice Guideline for LKD evaluation recommends using eGFR as a test to identify candidates who may not need a subsequent GFR assessment (63).

Previous research comparing eGFR and mGFR in living donors showed that suitable donors with a falsely low eGFR could have been accepted as well as inappropriate donors with a falsely high eGFR should have been rejected (54, 76).

Gaillard et al. (72) evaluated 2,733 donors from 11 French transplant centers. They examined whether relying on an eGFR rather than mGFR measurement alters the choice of potential living donors by comparing the effect of 4 equations (MDRD, CKD-EPI, Lund Malmö equation, and full age spectrum [FAS] equation) with mGFR as the reference method. Additionally, they studied the impact of using absolute or age-adapted GFR thresholds. They found that the CKD-EPI and FAS equations had the best performances and led to the lowest percentage of inappropriately evaluated candidates. Misclassification was more frequent when GFR adequacy was defined as an absolute threshold of 90 ml/ min/1.73 m^2 as compared with an age-adapted definition (26 and 5%, respectively). Accepting an absolute eGFR threshold of 90 ml/min/1.73 m^2, 1,804 potential donors were identified, compared with 2,648 when mGFR was interpreted using age-adjusted thresholds. They strongly suggest that mGFR should be the gold standard for donor evaluation, but in cases where eGFR is the only source of measurement, age-adapted GFR values estimated with either the CKD-EPI or FAS equations should be used.

Recently, Garg et al. (71) compared 1,412 donors in the performance of eGFR with CKD-EPI equation, 24-h CrCl, and the average of these 2 measurements (Avg [CrCl and eGFR] with mGFR by 125I-iothalamate as the gold standard). They found that 24-h CrCl overestimated iothalamate GFR (iGFR) in the entire cohort with an average bias of 2.2 ml/min/1.73 m^2. However, in men, CrCl overestimated mGFR by 6.7 ml/min/1.73 m^2, and in women, CrCl slightly underestimated GFR by 1 ml/min/1.73 m^2. eGFR underestimated iGFR with a median bias of −5.4 ml/min/1.73 m^2. Results were similar regardless of age and gender, however, in black potential donors, eGFR overestimated iGFR by 3.2 ml/min/1.73 m^2. Among the three testing GFR methods, median bias was significantly lower using Avg (CrCl and eGFR) at −1.0 ml/min/1.73 m^2. They concluded that these 2 GFR testing methods could provide a reference in clinical practice, as all transplant programs have access to them.

Renal Function After Donation

What happens with GFR after donation? With approximately 50% of the renal mass, the remaining kidney undergoes compensatory hypertrophy, and approximately 6 months after donation, GFR returns to 70% of the pre-donation values (79).

We have already addressed the literature regarding the risk of ESKD after donation but little attention has been paid to intermediate-risk factors that lead to it, such as DM, HTN, or impaired renal function. Ibrahim et al. (80) studied 3,956 LKDs followed for more than 40 years after donation. development

of ESKD, reduced GFR, reduced eGFR (< to 30, 45, and 60 ml/min/1.73 m^2) and proteinuria were analyzed. After a mean follow-up of 16.6 ± 11.9 years, 6.1% developed proteinuria after donation. This was associated with a higher body mass index (BMI) (*HR*, 1.10; 95% *CI*, 1.06–1.13; $p < 0.001$) and male gender (*HR*, 1.56; 95% *CI*, 1.18–2.05; $p < 0.001$). Regarding renal function, 35.6% had an eGFR <60 ml/min/1.73 m^2 at a median age of 56.6 years and a median time of 9.2 years from the donation. Risk factors associated with an eGFR < 60 were older age at donation, higher BMI, a higher baseline systolic blood pressure, and type 2 DM in the recipient. Additionally, 10.9% had an eGFR <45 ml/min/1.73 m^2, older age at donation, higher BMI, and higher baseline systolic blood pressure were their risk factors. Finally, 2.6% had an eGFR <30 ml/min/1.73 m^2 at a median age of 68.4 years and after a median time of 23.9 years after donation. This was associated with older age at donation and higher BMI. Interestingly, this study showed that a higher pre-donation eGFR and younger donor age was associated with better post-donation eGFR even after 40 years after donation. In multivariate time-dependent analysis, post-donation diabetes, new-onset hypertension, proteinuria, and eGFR < to 60 or 45 ml/min/1.73 m^2, were potent predictors of eGFR < 30 ml/min or ESKD.

In previous studies (16, 21), it has been shown that most of the donors developing ESKD donated their kidney to a relative. It is well known that relatives of a patients with ESKD have a higher risk of ESKD (81). Matas et al. (22) tried to determine whether donors having a relative with ESKD had a faster decline in eGFR compared with those without ESKD. They compared long-term post-donation eGFR trajectory for donors with (n = 1,245) vs. without (n = 757) a first-degree relative with ESKD. After adjusting for other patient factors, donors with a first-degree relative with ESKD had either a smaller increase or a larger decrease in eGFR—on average 0.20 ml/min/1.73 m^2/year (0.07–0.33) more than donors without a first-degree relative with ESKD, in a wide age range. The authors suggest that, for donors with and without a first-degree relative with ESRD, there is a steady increase in GFR for the first few year's post-donation. Although there was a small difference between the slope of GFR in donors with (vs. without) a first-degree relative with ESRD, neither group experienced a decline in GFR that would explain any increased incidence of ESRD.

A European study from the Netherlands, that we have already mentioned above (17), showed that in kidney donors after mean follow of more than 8 years, there were increase in serum creatinine of 26 μmol/l (95% *CI* 24–28), a decrease in eGFR of 27 ml/min/1.73 m^2 (95% *CI* −29 to −26), and an eGFR decline of 32% (95% *CI* 30–33) compared with non-donors. There were no differences between groups concerning ESKD. Recently, Agustine et al. (79) evaluated in a cohort of 34,505 LKDs from the Scientific Registry of Transplant Recipients (SRTR) database, factors associated with post-donation renal function and proteinuria. They found an overall median decline of eGFR of 31.1%. Nevertheless, 74% of donors older than 60 years had an eGFR < 60 ml/min 2 years after donation. At that time, they found that older donor age, male gender, black race, HTN, and a BMI > 25 kg/m^2 were associated with greater decline of eGFR. Although they found that incident proteinuria was independently associated with black race, male gender, and higher BMI, they could not find a correlation between proteinuria and eGFR decline 2 years after donation.

Finally, Gaillard et al. (82) in 2021 studied 1,825 French LKDs. After a mean follow-up of almost 6 years, they found that in donors younger than 45 years post-donation eGFR, absolute- and relative-eGFR variation were not different among the three groups, normal for age (Sage), higher than 90 (S90), or 80(S80) ml/min/1.73 m^2. However, for older donors, eGFR after donation was higher in S90 than in S80 or Sage. They concluded that donors with a normal eGFR for age (Sage) are older than donors with an eGFR ≥ 90 ml/min/1.73 m^2 (S90). Differences in GFR after donation are partly attributable to this age difference. They suggest a depth screening for all donor candidates with a normal eGFR for age.

CONCLUSIONS

Currently, the best way to assess the kidney function of donor remains a matter of discussion. The available guidelines for kidney donation are purely based on opinion, and they are not evidence-based. Several methods are clinically available and recommended for living donor evaluation, but each has its drawbacks. Moreover, most of them have been only validated for CKD population. mGFR remains the gold standard, but it is not available worldwide. Therefore, GFR at donation should be considered in the context of normal GFR level based on gender and age. In our experience and that of others, a combination of 2 methods, such as a CrCl and eGFR, may be more clinically useful. Transplant programs are responsible for developing a threshold to accept or deny a living donor based on lifetime risk of kidney failure and apply them uniformly.

Finally, renal function recovery is variable and data suggests that GFR recovery, and post-donation GFR, is associated with donor age, BMI, gender, HTN, and family history.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

REFERENCES

1. Zhang QL, Rothenbacher, D. Prevalence of chronic kidney disease in population-based studies: Systematic review. *BMC Public Health.* (2008) 8:1–3. doi: 10.1186/1471-2458-8-117
2. Liyanage T, Ninomiya T, Jha V, Neal B, Patrice HM, Okpechi I, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. *Lancet.* (2015) 385:1975–82. doi: 10.1016/S0140-6736(14)61601-9
3. Manns BJ, Mendelssohn DC, Taub KJ. The economics of end-stage renal disease care in Canada: incentives and impact on delivery of care. *Int*

J Health Care Finance Econ. (2007) 7:149–69. doi: 10.1007/s10754-007-9022-y
4. Klarenbach S, Gill JS, Knoll G, Caulfield T, Boudville N, Prasad GV, et al. Donor Nephrectomy Outcomes Research (DONOR) Network. Economic consequences incurred by living kidney donors: a Canadian multi-center prospective study. *Am J Transplant.* (2014) 14:916–22. doi: 10.1111/ajt.12662
5. Canadian Institute for Health Information. Report: CORR 2016. (2016). Available online at: https://www.cihi.ca/en/organ-replacements/corrannual-report. (accessed June 1, 2018).
6. Laupacis A, Keown P, Pus N, Krueger H, Ferguson B, Wong C, et al. A study of the quality of life and cost-utility of renal transplantation. *Kidney Int.* (1996) 50:235–42. doi: 10.1038/ki.1996.307
7. Klarenbach SW, Tonelli M, Chui B, Manns BJ. Economic evaluation of dialysis therapies. *Nat Rev Nephrol.* (2014) 10:644–52. doi: 10.1038/nrneph.2014.145
8. Marinovich S, Bisigniano L, Hansen Krogh D, Celia E, Tagliafichi V, Rosa Diez G, et al. *Registro Argentino de Diálisis Crónica SAN-INCUCAI 2019.* Sociedad Argentina de Nefrología e Instituto Nacional Central Único Coordinador de Ablación e Implante Buenos Aires, Argentina. (2020).
9. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med*. (1999) 341:1725–30 doi: 10.1056/NEJM199912023412303
10. Reese PP, Feldman HI, McBride MA, Anderson K, Asch DA, Bloom RD. Substantial variation in the acceptance of medically complex live kidney donors across US renal transplant centers. *Am J Transplant.* (2008) 8:2062–70. doi: 10.1111/j.1600-6143.2008.02361.x
11. Sergev DL, Muzaale AD, Caffo BS, Mehta SH, Singer AL, Taranto SE, et al. Perioperative mortality and long-term survival following live kidney donation. *JAMA*. (2010) 303:959–66. doi: 10.1001/jama.2010.237
12. Steiner CA, Bass EB, Talamini MA, Pitt HA, Steinberg EP. Surgical rates and operative mortality for open and laparoscopic cholecystectomy in Maryland. *N Engl J Med*. (1994) 330:403–8. doi: 10.1056/NEJM199402103300607
13. Birkmeyer JD, Siewers AE, Finlayson EV, Stukel TA, Lucas FL, Batista I, et al. Hospital volume and surgical mortality in the United States. *N Engl J Med.* (2002) 346:1128–37. doi: 10.1056/NEJMsa012337
14. Fehrman-Ekholm I, Elinder CG, Stenbeck M, Tydén G, Groth CG. Kidney donors live longer. *Transplantation.* (1997) 64:976–8. doi: 10.1097/00007890-199710150-00007
15. Ibrahim HN, Foley R, Tan L, Rogers T, Bailey RF, Guo H, et al. Long-term consequences of kidney donation. *N Engl J Med.* (2009) 360:459–69. doi: 10.1056/NEJMoa0804883
16. Mjøen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Øyen O, et al. Long-term risks for kidney donors. *Kidney Int.* (2014) 86:162–7. doi: 10.1038/ki.2013.460
17. Janki S, Dehghan A, van de Wetering J, Steyerberg EW, Klop KWJ, Kimenai HJAN, et al. Long-term prognosis after kidney donation: a propensity score matched comparison of living donors and non-donors from two population cohorts. *Eur J Epidemiol.* (2020) 35:699–707. doi: 10.1007/s10654-020-00647-y
18. Okamoto M, Akioka K, Nobori S, Ushigome H, Kozaki K, Kaihara S, et al. Short-and long-term donor outcomes after kidney donation: analysis of 601 cases over a 35-year period at Japanese single center. *Transplantation.* (2009) 87:419–23. doi: 10.1097/TP.0b013e318192dc95
19. Fournier C, Pallet N, Cherqaoui Z, Pucheu S, Kreis H, Méjean A, et al. Very long-term follow-up of living kidney donors. *Transpl Int*. (2012) 25:385–90. doi: 10.1111/j.1432-2277.2012.01439.x
20. Matas AJ, Hays RE, Ibrahim HN. A case-based analysis of whether living related donors listed for transplant share ESRD causes with their recipients. *Clin J Am Soc Nephrol.* (2017) 12:663–8. doi: 10.2215/CJN.11421116
21. Muzaale AD, Massie AB, Wang MC, Montgomery RA, McBride MA, Wainright JL, et al. Risk of end-stage renal disease following live kidney donation. *JAMA*. (2014) 311:579–86. doi: 10.1001/jama.2013.285141
22. Matas AJ, Vock DM, Ibrahim HN. GFR. ≤25 years postdonation in living kidney donors with (vs without) a first-degree relative with ESRD. *Am J Transplant.* (2018) 18:625–31. doi: 10.1111/ajt.14525
23. LaPointe Rudow D, Warburton KM. Selection and postoperative care of the living donor. *Med Clin North Am.* (2016) 100:599–611. doi: 10.1016/j.mcna.2016.01.009
24. O'Keeffe LM, Ramond A, Oliver-Williams C, Willeit P, Paige E, Trotter P, et al. Mid- and long-term health risks in living kidney donors: a systematic review and meta-analysis. *Ann Intern Med.* (2018) 168:276–84. doi: 10.7326/M17-1235
25. Kasiske BL, Anderson-Haag T, Israni AK, Kalil RS, Kimmel PL, Kraus ES, et al. prospective controlled study of living kidney donors: three-year follow-up. *Am J Kidney Dis.* (2015) 66:114–24. doi: 10.1053/j.ajkd.2015.01.019
26. Levey AS. Measurement of renal function in chronic renal disease. *Kidney Int.* (1990) 38:167–84. doi: 10.1038/ki.1990.182
27. Pérez Loredo J, Lavorato CA, Negri AL. Tasa de filtración glomerular medida y estimada. Numerosos métodos de medición (Parte I). *Rev Nefrol Dial Traspl*. (2017) 35:153–64. Available online at: https://www.revistarenal.org.ar/index.php/rndt/article/view/34
28. Eurotransplant Statistical Report 2017. Available online at https://www.eurotransplant.org/cms/index.php?page=sr2017. (accessed February 9, 2019).
29. Ribstein J, du Cailar G, Mimran A. Combined renal effects of overweight and hypertension. *Hypertension.* (1995) 26:610–5. doi: 10.1161/01.HYP.26.4.610
30. Nielsen S, Rehling M, Schmitz A, Mogensen CE. Validity of rapid estimation of glomerular filtration rate in type 2 diabetic patients with normal renal function. *Nephrol Dial Transplant.* (1999) 14:615–9. doi: 10.1093/ndt/14.3.615
31. McLeay SC, Morrish GA, Kirkpatrick CM, Green B. Encouraging the move towards predictive population models for the obese using propofol as a motivating example. *Pharm Res.* (2009) 26:1626–34. doi: 10.1007/s11095-009-9873-7
32. Documento de consenso sobre la Enfermedad Renal Crónica. Available online at: http://www.senefro.org/modules/news/images/v._5.doc_consenso_final___131212_copy1.pdf. (accessed Noviembre 27, 2012)
33. Thomassen SA, Johannesen IL, Erlandsen EJ, Abrahamsen J, Randers E. Serum cystatin C as a marker of the renal function in patients with spinal cord injury. *Spinal Cord.* (2002) 40:524–8. doi: 10.1038/sj.sc.3101320
34. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* (2012) 367:20–9. doi: 10.1056/NEJMoa1114248
35. Filler G, Priem F, Lepage N, Sinha P, Vollmer I, Clark H, et al. Beta-trace protein, cystatin C, beta(2)-microglobulin, and creatinine compared for detecting impaired glomerular filtration rates in children. *Clin Chem.* (2002) 48:729–36. doi: 10.1093/clinchem/48.5.729
36. Shannon JA, Smith HW. The excretion of inulin, xylose and urea by normal and phlorizinized man. *J Clin Invest.* (1935) 14:393–401. doi: 10.1172/JCI100690
37. Marsh D. Frasier C: Reliability of inulin for determining volume flow in rat renal cortical tubules. *Am J Physiol.* (1965) 209:283–6. doi: 10.1152/ajplegacy.1965.209.2.283
38. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco AL, De Jong PE, et al. Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int. Suppl.* (2013) 3:1–150.
39. Davies DF, Shock NW. The variability of measurement of insulin and diodrast tests of kidney function. *J Clin Invest.* (1950) 29:491–5. doi: 10.1172/JCI102285
40. Soveri I, Berg UB, Björk J, Elinder CG, Grubb A, Mejare I, et al. Review Group. Measuring GFR: a systematic review. *Am J Kidney Dis.* (2014) 64:411–24. doi: 10.1053/j.ajkd.2014.04.010
41. Anderson CF, Sawyer TK, Cutler RE. Iothalamate sodium I 125 vs cyanocobalamin Co 57 as a measure of glomerular filtration rate in man. *JAMA.* (1968) 204:653–6. doi: 10.1001/jama.1968.03140210007002
42. Odlind B, Hällgren R, Sohtell M, Lindström B. Is 125I iothalamate an ideal marker for glomerular filtration? *Kidney Int.* (1985) 27:9–16. doi: 10.1038/ki.1985.3
43. Botev R, Mallié JP, Wetzels JF, Couchoud C, Schück O. The clinician and estimation of glomerular filtration rate by creatinine-based formulas: current limitations and quo vadis. *Clin J Am Soc Nephrol.* (2011) 6:937–50. doi: 10.2215/CJN.09241010
44. Rehling M, Møller ML, Thamdrup B, Lund JO, Trap-Jensen J. Simultaneous measurement of renal clearance and plasma clearance of 99mTc-labelled diethylenetriaminepenta-acetate, 51Cr-labelled ethylenediaminetetra-acetate and inulin in man. *Clin Sci (Lond).* (1984) 66:613–9. doi: 10.1042/cs0660613

45. De Santo NG, Anastasio P, Cirillo M, Santoro D, Spitali L, Mansi L, et al. Measurement of glomerular filtration rate by the 99mTc-DTPA renogram is less precise than measured and predicted creatinine clearance. *Nephron.* (1999) 81:136–40. doi: 10.1159/000045268
46. Olsson B, Aulie A, Sveen K, Andrew E. Human pharmacokinetics of iohexol: a new nonionic contrast medium. *Invest Radiol.* (1983) 18:177–82. doi: 10.1097/00004424-198303000-00015
47. Skinnemoen K. Physicochemical properties and degree of protein binding of iopentol. *Acta Radiol Suppl.* (1987) 370:33–6.
48. Sterner G, Frennby B, Mansson S, Nyman U, Van Westen D, Almén T. Determining 'true' glomerular filtration rate in healthy adults using infusion of inulin and comparing it with values obtained using other clearance techniques or prediction equations. *Scand J Urol Nephrol.* (2008) 42:278–85. doi: 10.1080/00365590701701806
49. Levey AS, Greene T, Schluchter MD, Cleary PA, Teschan PE, Lorenz RA, et al. Glomerular filtration rate measurements in clinical trials. Modification of diet in renal disease study group and the diabetes control and complications trial research group. *J Am Soc Nephrol.* (1993) 4:1159–71. doi: 10.1681/ASN.V451159
50. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, et al. more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
51. Stevens LA, Manzi J, Levey AS, Chen J, Deysher AE, Greene T, et al. Impact of creatinine calibration on performance of GFR estimating equations in a pooled individual patient database. *Am J Kidney Dis.* (2007) 50:21–35. doi: 10.1053/j.ajkd.2007.04.004
52. Bhuvanakrishna T, Blake GM, Hilton R, Burnapp L, Sibley-Allen C, Goldsmith D. Comparison of estimated GFR and measured GFR in prospective living kidney donors. *Int Urol Nephrol.* (2015) 47:201–8. doi: 10.1007/s11255-014-0859-y
53. Murata K, Baumann NA, Saenger AK, Larson TS, Rule AD, Lieske JC. Relative performance of the MDRD and CKD-EPI equations for estimating glomerular filtration rate among patients with varied clinical presentations. *Clin J Am Soc Nephrol.* (2011) 6:1963–72. doi: 10.2215/CJN.02300311
54. Tent H, Rook M, Stevens LA, van Son WJ, van Pelt LJ, Hofker HS, et al. Renal function equations before and after living kidney donation: a within-individual comparison of performance at different levels of renal function. *Clin J Am Soc Nephrol.* (2010) 5:1960–8. doi: 10.2215/CJN.08761209
55. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int.* (2004) 65:1416–21. doi: 10.1111/j.1523-1755.2004.00517.x
56. Herget-Rosenthal S, Bökenkamp A, Hofmann W. How to estimate GFR-serum creatinine, serum cystatin C or equations? *Clin Biochem.* (2007) 40:153–61. doi: 10.1016/j.clinbiochem.2006.10.014
57. Meeusen JW, Rule AD, Voskoboev N, Baumann NA, Lieske JC. Performance of cystatin C- and creatinine-based estimated glomerular filtration rate equations depends on patient characteristics. *Clin Chem.* (2015) 61:1265–72. doi: 10.1373/clinchem.2015.243030
58. Almén MS, Björk J, Nyman U, Lindström V, Jonsson M, Abrahamson M, et al. Shrunken pore syndrome is associated with increased levels of atherosclerosis-promoting proteins. *Kidney Int Rep.* (2018) 4:67–79. doi: 10.1016/j.ekir.2018.09.002
59. Grubb A, Lindström V, Jonsson M, Bäck SE, Åhlund T, Rippe B, et al. Reduction in glomerular pore size is not restricted to pregnant women. Evidence for a new syndrome: 'Shrunken pore syndrome'. *Scand J Clin Lab Invest.* (2015) 75:333–40. doi: 10.3109/00365513.2015.1025427
60. Dardashti A, Nozohoor S, Grubb A, Bjursten H. Shrunken Pore Syndrome is associated with a sharp rise in mortality in patients undergoing elective coronary artery bypass grafting. *Scand J Clin Lab Invest.* (2016) 76:74–81. doi: 10.3109/00365513.2015.1099724
61. Christensson A, Grubb A, Molvin J, Holm H, Gransbo K, Tasevska-Dinevska G, et al. The shrunken pore syndrome is associated with declined right ventricular systolic function in a heart failure population - the HARVEST study. *Scand J Clin Lab Invest.* (2016) 76:568–74. doi: 10.1080/00365513.2016.1223338
62. González-Rinne A, Luis-Lima S, Escamilla B, Negrín-Mena N, Ramírez A, Morales A, et al. Impact of errors of creatinine and cystatin C equations in the selection of living kidney donors. *Clin Kidney J.* (2019) 12:748–55. doi: 10.1093/ckj/sfz012
63. Lentine KL, Kasiske BL, Levey AS, Adams PL, Alberú J, Bakr MA, et al. KDIGO clinical practice guideline on the evaluation and care of living kidney donors. *Transplantation.* (2017) 101:S1–S109. doi: 10.1097/TP.0000000000001769
64. Grams ME, Garg AX, Lentine KL. Kidney-failure risk projection for the living kidney-donor candidate. *N Engl J Med.* (2016) 374:2094–5. doi: 10.1056/NEJMc1603007
65. British Transplantation Society. BTS/RA Living Donor Kidney Transplantation Guidelines (2018). Available online at https://bts.org.uk/wp-content/uploads/2018/07/FINAL_LDKT-guidelines_June-2018.pdf. (accessed February 9, 2019).
66. Peters AM, Perry L, Hooker CA, Howard B, Neilly MD, Seshadri N, et al. Extracellular fluid volume and glomerular filtration rate in 1878 healthy potential renal transplant donors: effects of age, gender, obesity and scaling. *Nephrol Dial Transplant.* (2012) 27:1429–37. doi: 10.1093/ndt/gfr479
67. Richardson R, Connelly M, Dipchand C, Garg AX, Ghanekar A, Houde I, et al. Protocols working group of the canadian blood services' living donation advisory committee: kidney paired donation protocol for participating donors 2014. *Transplantation.* (2015) 99:S1–S88. doi: 10.1097/TP.0000000000000918
68. Organ Procurement and Transplantation Network. Available online at: https://optn.transplant.hrsa.gov/media/1200/optn_policies.pdf#nameddest=Policy_14.
69. Gaston R, Wadström J. *Living Donor Kidney Transplantation: Current Practices, Emerging Trends and Evolving Challenges.* (1st ed.). CRC Press. (2005). doi: 10.1201/b14909
70. Organ Procurement and Transplantation Network. Available online at: https://optn.transplant.hrsa.gov/resources/guidance/guidance-for-the-development-of-program-specific-living-kidney-donor-medical-evaluation-protocols/.
71. Garg N, Snyder G, Li J, Mandelbrot D, Poggio ED. Performance of creatinine clearance and estimated GFR in assessing kidney function in living donor candidates. *Transplantation.* (2020) 104:575–82. doi: 10.1097/TP.0000000000002797
72. Gaillard F, Courbebaisse M, Kamar N, Rostaing L, Jacquemont L, Hourmant M, et al. Impact of estimation versus direct measurement of predonation glomerular filtration rate on the eligibility of potential living kidney donors. *Kidney Int.* (2019) 95:896–904. doi: 10.1016/j.kint.2018.11.029
73. Gaillard F, Courbebaisse M, Kamar N, Rostaing L, Del Bello A, Girerd S, et al. The age-calibrated measured glomerular filtration rate improves living kidney donation selection process. *Kidney Int.* (2018) 94:616–24. doi: 10.1016/j.kint.2018.05.016
74. Mandelbrot DA, Pavlakis M, Danovitch GM, Johnson SR, Karp SJ, Khwaja K, et al. The medical evaluation of living kidney donors: a survey of US transplant centers. *Am J Transplant.* (2007) 7:2333–43. doi: 10.1111/j.1600-6143.2007.01932.x
75. Gaillard F, Legendre C, White CA, GFR. Assessment of living kidney donors candidates. *Transplantation.* (2019) 103:1086–93. doi: 10.1097/TP.0000000000002620
76. Garg N, Poggio E, Mandelbrot D. The evaluation of kidney function in living kidney donor candidates. *Kidney360.* (2021) 9:1523–1530. doi: 10.34067/KID.0003052021
77. Gaillard F, Flamant M, Lemoine S, Baron S, Timsit MO, Eladari D, et al. Estimated or measured GFR in living kidney donors work-up? *Am J Transplant.* (2016) 16:3024–32. doi: 10.1111/ajt.13908
78. Huang N, Foster MC, Lentine KL, Garg AX, Poggio ED, Kasiske BL, et al. Estimated GFR for living kidney donor evaluation. *Am J Transplant.* (2016) 16:171–80. doi: 10.1111/ajt.13540
79. Augustine JJ, Arrigain S, Mandelbrot DA, Schold JD, Poggio ED. factors associated with residual kidney function and proteinuria after living kidney donation in the United States. *Transplantation.* (2021) 105:372–81. doi: 10.1097/TP.0000000000003210
80. Ibrahim HN, Foley RN, Reule SA, Spong R, Kukla A, Issa N, et al. Renal function profile in white kidney donors: the first 4 decades.

J Am Soc Nephrol. (2016) 27:2885–93. doi: 10.1681/ASN.2015091018

81. Bergman S, Key BO, Kirk KA, Warnock DG, Rostant SG. Kidney disease in the first-degree relatives of African-Americans with hypertensive end-stage renal disease. *Am J Kidney Dis.* (1996) 27:341–6. doi: 10.1016/S0272-6386(96)90356-X
82. Gaillard F, Jacquemont L, Lazareth H, Albano L, Barrou B, Bouvier N, et al. Living kidney donor evaluation for all candidates with normal estimated GFR for age. *Transpl Int.* (2021) 34:1123–33. doi: 10.1111/tri.13870

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

MINI REVIEW
published: 03 February 2022
doi: 10.3389/fmed.2021.769734

Prematurity and Low Birth Weight in Neonates as a Risk Factor for Obesity, Hypertension, and Chronic Kidney Disease in Pediatric and Adult Age

Maria Agostina Grillo[1,2], *Gonzalo Mariani*[1,3,4] *and Jorge R. Ferraris*[1,2,5]*

[1] Pediatric Department Hospital Italiano de Buenos Aires, Buenos Aires, Argentina, [2] Pediatric Nephrology Division, Buenos Aires, Argentina, [3] Neonatology Division, Buenos Aires, Argentina, [4] Instituto Universitario Hospital Italiano de Buenos Aires, Buenos Aires, Argentina, [5] Pediatric Department, Universidad de Buenos Aires, Buenos Aires, Argentina

Low weight at birth may be due to intrauterine growth restriction or premature birth. Preterm birth is more common in low- and middle-income countries: 60% of preterm birth occur in sub-Saharan African or South Asian countries. However, in some higher-income countries, preterm birth rates appear to be increasing in relation to a reduction in the lower threshold of fetal viability. The cutoff is at 22–23 weeks, with a birth weight of approximately 500 g, although in developed countries such as Japan, the viability cutoff described is 21–22 weeks. There is evidence of the long-term consequences of prenatal programming of organ function and its relationship among adult diseases, such as hypertension (HT), central obesity, diabetes, metabolic syndrome, and chronic kidney disease (CKD). Premature delivery before the completion of nephrogenesis and intrauterine growth restriction leads to a reduction in the number of nephrons that are larger due to compensatory hyperfiltration and hypertrophy, which predisposes to the development of CKD in adulthood. In these patients, the long-term strategies are early evaluation and therapeutic interventions to decrease the described complications, by screening for HT, microalbuminuria and proteinuria, ultrasound monitoring, and renal function, with the emphasis on preventive measures. This review describes the effects of fetal programming on renal development and the risk of obesity, HT, and CKD in the future in patients with low birth weight (LBW), and the follow-up and therapeutic interventions to reduce these complications.

Keywords: preterm neonates, low birth weight, chronic kidney disease, arterial hypertension, fetal programming, extrauterine growth restriction, intrauterine growth restriction, small gestational age

OPEN ACCESS

Edited by:
Ana Cusumano, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Argentina

Reviewed by:
Brian Duncan Tait, The University of Melbourne, Australia
Karel Allegaert, University Hospitals Leuven, Belgium
Jessica Briffa, The University of Melbourne, Australia

***Correspondence:**
Jorge R. Ferraris
jorge.ferraris@hospitalitaliano.org.ar

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *02 September 2021*
Accepted: *23 November 2021*
Published: *03 February 2022*

Citation:
Grillo MA, Mariani G and Ferraris JR (2022) Prematurity and Low Birth Weight in Neonates as a Risk Factor for Obesity, Hypertension, and Chronic Kidney Disease in Pediatric and Adult Age. Front. Med. 8:769734. doi: 10.3389/fmed.2021.769734

INTRODUCTION

Preterm birth affects ∼11% of births worldwide. The availability of new therapeutics and the increasing complexity of neonatal intensive care units have allowed the survival of infants born at 22 or 23 weeks with birth weights close to 500 g (1–3). The annual prevalence of prematurity in Argentina is between 8 and 9% (4). In this article, we define preterm newborns (PTNs) as those born before 37 week gestational age (GA); small

for gestational age (SGA) as neonates with a birth weight less than the 10th percentile for their GA; low birth weight (LBW) and very low birth weight (VLBW) as those with birth weight <2,500 and 1,500 g, respectively, and extremely low birth weight (ELBW) as those with birth weight <1.0 kg.

Preterm newborns and SGA are particularly vulnerable to the development of hypertension (HT) and chronic kidney disease (CKD). In the former, there is premature exposure to the conditions of extrauterine life, in organs that are not yet prepared for it, where the premature arrest of the development of the vascular tree results in stiffer and narrower arteries, which predisposes to glomerular and endothelial damage, structural alterations due to glomerular hyperfiltration, and increased systolic blood pressure (SBP) in children and adults (5, 6). Preterm infants may also have either an appropriate birth weight for GA or maybe SGA if they experienced superimposed growth restriction. Such growth restriction *per se* is also associated with programming effects in the kidney (7). In SGA infants who have had intrauterine growth restriction (percentile drop throughout pregnancy as a consequence of an alteration in placental circulation), exposure to intrauterine stress generates an altered "fetal programming," inducing changes at the molecular level and in the functioning of systems, with alterations in renal growth and a decrease in the number of nephrons, which would increase the incidence of HT, CKD, and the risk of metabolic alterations, such as insulin resistance. Pregnancies affected by maternal HT have greater short-term fetal complications, such as fetal death and SGA, as a consequence of placental insufficiency due to preeclampsia (8, 9). The association between preeclampsia and SGA is based on abnormal placental development and decreased placental perfusion, secondary to alteration of the maternal spiral arteries, with spontaneous vasoconstriction of the arteries and placental ischemia, reperfusion-type injury, and oxidative stress (10, 11) (**Figure 1**).

PATHOPHYSIOLOGY

Nephrogenesis ends at 36 weeks and the reduction in the number of nephrons has consequences for renal health. The number of glomeruli in the normal human embryo increases from week 10, reaches its largest increase between 18 and 32 weeks and is completed between 32 and 36 weeks (12). Each normal kidney has an average of 600–800 thousand glomeruli. Birth weight correlates with the number of glomeruli, estimating an additional 2,32,217 nephrons in each kidney for each 1 kg of birth weight (13, 14). The number of nephrons is reduced by factors that restrict intrauterine growth: micronutrient deficiencies, infections, hypoxia, drugs (nephrotoxic or not, such as beta-lactams), maternal hyperglycemia, glucocorticoids, smoking, or alcohol consumption during pregnancy. The nephron endowment reached at birth will be the one with which the individual will spend the rest of his or her life (13–16).

According to Brenner's hyperfiltration theory (17, 18), humans with a decreased nephron endowment can maintain a normal GFR as individual nephron hypertrophy to increase the total surface area available for renal work. Over time, this adaptive response becomes harmful. The increased glomerular surface area leads to sodium retention and systemic HT and glomerular hyperfiltration disrupts renal autoregulatory mechanisms generating intraglomerular HT (19). These processes render the nephrons sclerotic and this leads to a further decrease in the number of nephrons that reduces the filtration surface, and the remaining nephrons must hypertrophy, manifesting with microalbuminuria and then proteinuria as surrogates of hyperfiltration (12). As a consequence, arterial and glomerular HT is produced, generating glomerulosclerosis, further reducing the number of nephrons (**Figure 2**). In the terminal phases of CKD, widespread deposition of extracellular matrix in the renal interstitium is recognized as a final common pathway for nephron destruction, resulting from the maladaptive repair of damaged nephrons (20).

Whereas growth restriction increases disease risk in all individuals, often a second hit is required to unmask "programmed" impairments. Programmed disease outcomes are demonstrated more commonly in male offspring compared with females, with these sex-specific outcomes partly attributed to different placenta-regulated growth strategies of the male and female fetus. An extremely common and severe "second hit" for women, known to unmask a variety of conditions in adult life, is pregnancy; it is the greatest physiological "stress test" that a woman can experience in her life. Females who were born small are at an increased risk of pregnancy complications (preeclampsia, gestational diabetes, HT, thyroid, and liver and kidney diseases). The fetus that developed in the womb may also have been exposed to suboptimal conditions and may be programmed to develop the disease in later life, consequences of being born small due to uteroplacental insufficiency. Male fetuses grow at a faster rate than do females and this accelerated growth trajectory makes male fetuses more vulnerable during disturbed pregnancies, with less favorable outcomes occurring throughout the life course of the individual. These sexually dimorphic adaptations are regulated by the placenta. In animal models (e.g., rats), uteroplacental insufficiency results in LBW and programs sex-specific offspring dysfunction and deficits that affect males more than females: development of increased SBP in adult life. This is despite both sexes having decreased nephron number, earlier glomerular hypertrophy, and impaired glucose tolerance, and reduced insulin secretion. Although women are generally less susceptible to programmed disease development, under the physiological demands of pregnancy, various disease states are often unmasked (21).

EFFECTS OF PREMATURITY ON NEPHROGENESIS

The number of glomeruli is significantly lower in all groups of preterm infants (22). As 60% of nephrons are formed during the third trimester, children born preterm have a significantly lower number of nephrons at birth, which does not catch up adequately postnatally (7). The progression of postnatal nephrogenesis, evaluated in autopsies in preterm infants, evidenced the persistence of glomerulogenesis after

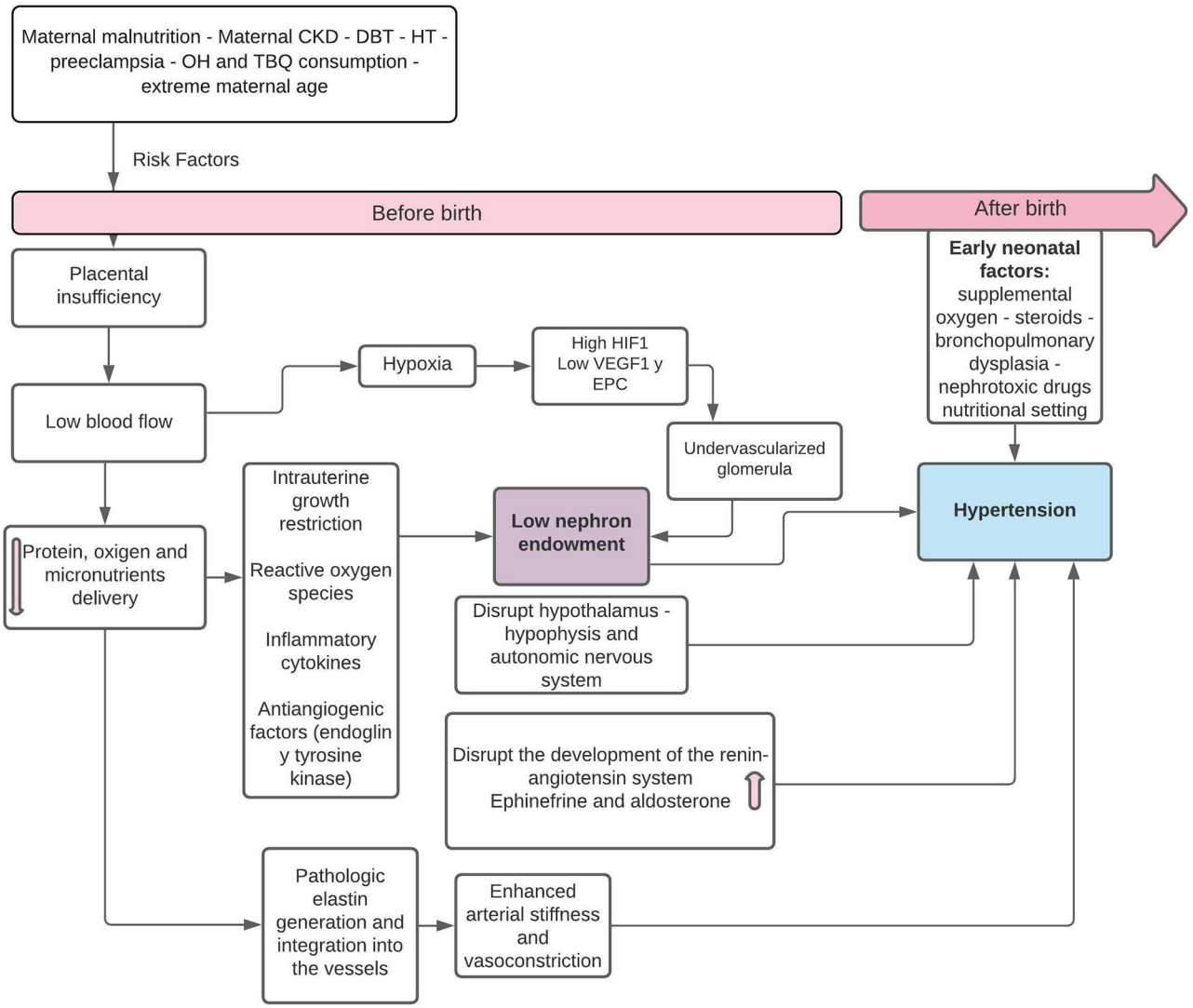


FIGURE 1 | Pathophysiology of fetal "programming" in SGA infants in relation to the decrease in the number of nephrons and postnatal HT. HIF-1, hypoxia-induced factor-1; SGA, small for gestational age; VEGF, vascular endothelial growth factor; EPC, neonatal endothelial progenitor cells. Modified from Stritzke et al. (9) and Crump et al. (11).

birth but altered and with a gradual decrease as postnatal age progressed. Neonates more than 40 days old with acute kidney injury (AKI) showed lower glomerular counts, unlike those with longer survival and without renal failure, but had glomerulomegaly as a compensatory mechanism (23). However, the development of animal models (surgical renal ablation, renal fibrosis, or others) would be important for the study of the effects of prematurity on nephrogenesis (24).

The causes underlying a reduced number of nephrons in an individual are both genetic and environmental. Ongoing interaction between genes and the environment from prenatal to adult life will contribute toward defining the renal potential of an individual. Signaling molecules and transcription factors have been implicated in determining segmental nephron identity and functional differentiation. Whereas some of these genes (p53 gene family, hepatocyte nuclear factor-1) promote the terminal epithelial differentiation fate, others (Notch, Brn-1, IRX, KLF4, and Foxi1) regulate the differentiation of specific nephron segments and cellular types (25). Moreover, epigenetic changes, characterized by alterations in chromatin structure, lead to stable and potentially hereditable changes in gene expression. In particular, DNA methylation has been strongly implicated in fetal renal development and disease (25).

Studies in clinically stable PTN demonstrate that plasma creatinine correlates with GA. Plasma creatinine at birth reflects tubular reabsorption of creatinine. Creatinine increases in the first 36–92 h of life and then gradually decreases. In PTN with GA <32 weeks, the increase in plasma creatinine is greater and the decrease more gradual (being greater in those born with <28 weeks), probably due to a slow progression of glomerular function and tubular creatinine reabsorption (26). Tubular creatinine reabsorption may be a physiological phenomenon in

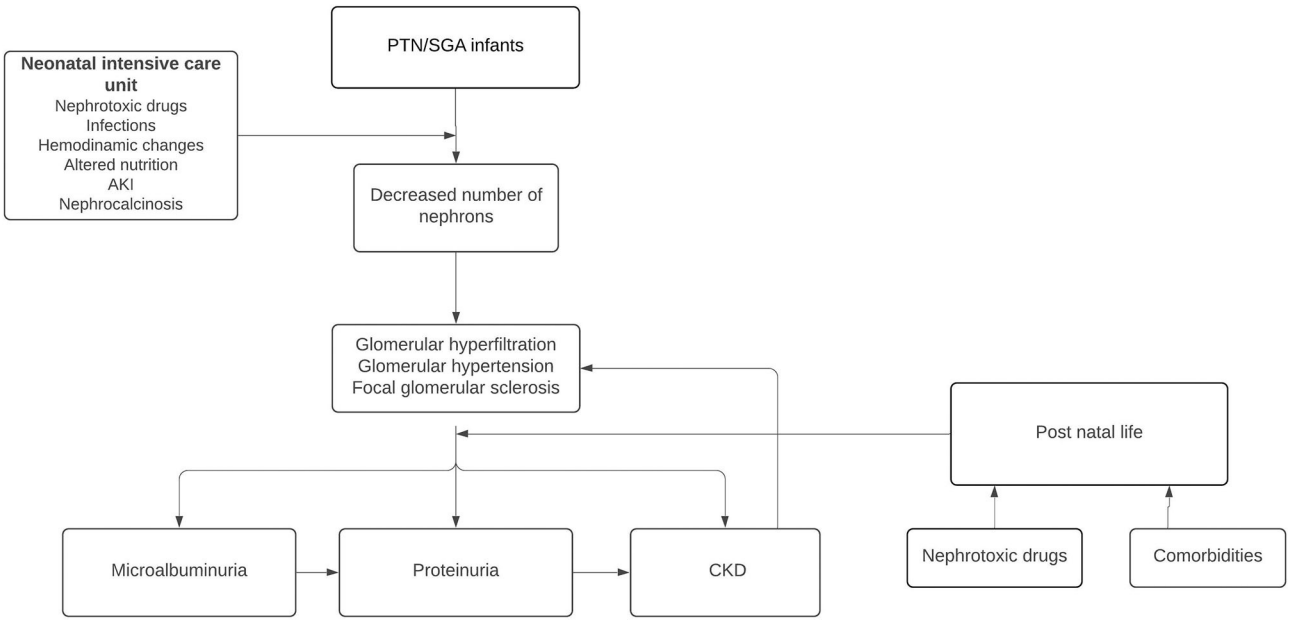


FIGURE 2 | Pathophysiological consequences of altered nephrogenesis in preterm newborns (PTN) and SGA infants.

the "immature" kidney due to slow urinary flow and increased creatinine leakage along the immature tubular structures (19).

Preterm newborns may present with AKI events (8–24%) secondary to renal hypoperfusion, asphyxia, respiratory distress syndrome, nephrotoxic drugs exposure (prenatal or postnatal), and infections. In addition, PTNs who are SGA are more vulnerable to renal injury, as SGA has greater nephron depletion and renal dysfunction (22). Decreased glomerular filtration rate (GFR) and increased microalbuminuria have been observed in children and adults who were PTN and SGA, compared with adult PTN but with adequate weight for GA (22).

Epidemiologic studies have shown that incomplete recovery from episodes of AKI constitutes a risk factor for progressive CKD, and CKD, in turn, increases susceptibility to AKI: the proximal tubule, therefore, becomes a primary target of injury and progression of CKD (20). Preterm and critically ill newborns are predisposed to developing AKI because of renal function immaturity and incomplete nephrogenesis in the early postnatal period, which can be irreversibly impaired by drug exposure, and cellular injury to glomeruli or tubules, which may impair repair capacity and increase susceptibility to renal disease later in life (7). In the US, 40% of ICU neonates experienced AKI, it was found that AKI was only recorded in the discharge summary in 13.5% of infants, and none were referred for nephrology follow-up (27). This study illustrated the lack of awareness of the potential long-term impact of neonatal AKI.

On the other hand, PTNs are at risk of extrauterine growth restriction (EUGR), defined as growth below the 10th percentile of growth expectancy, generating consequently greater alterations in nephrogenesis and renal function in adulthood. A lower GFR was evidenced in children examined at 7 years of age (preterm <30 weeks whether SGA or EUGR) compared with children with adequate prenatal and postnatal growth (22). Both intra- and extrauterine growth restrictions were associated with reduced GFR. However, rapid "catchup" growth (i.e., an upward crossing of weight centiles) or increase in BMI leads to the development of higher blood pressure, insulin resistance, and cardiovascular risk already in childhood. These findings are most marked in those who were born small and became relatively larger (28).

Along the same lines, in children aged 1–7 years with a history of PTN and SGA, decreased GFR (78 ± 26.8 ml/min/1.73 m^2), microalbuminuria (85 ± 187 mg/gr), increased SBP in 21%, and diastolic blood pressure (DBP) in 37% of patients were observed, with mean SBP and DBP between 10 and 15 mmHg above the mean of healthy term newborns. Renal volume increased until 2.5 years of age and then decreased, implying glomerular hypertrophy in the first stage and then possibly glomerular sclerosis (29).

LONG-TERM CONSEQUENCES IN THE KIDNEY OF THE PRETERM AND SGA NEONATE

Early renal complications are related to immaturity in tubular function (tubulopathy of prematurity), presenting inadequate free water management, electrolyte and acid-base imbalance, and mineral and protein losses (30). The increase in GFR that occurs from birth is accompanied by a "parallel" increase in tubular functions to avoid water and solute losses through urine. The activity of the Na+ -K+ -ATPase pump is proportional to GA, which explains the lower reabsorption capacity in PTN <32 weeks (31). Insensible water losses increase in inverse relation

to GA. The kidney is in frank natriuresis, inversely proportional to GA, and in PTN <35 weeks, the tubule is unable to conserve sodium. Early onset neonatal hyponatremia in PTNs is secondary to excess water intake associated with increased antidiuretic hormone secretion (30, 31). Serum bicarbonate is lower in PTN (with renal threshold 18 mEq/L) or weight <1,300 g (renal threshold 14 mEq/L); the mechanisms that regulate bicarbonate absorption and secretion have progressive maturation (31).

Late complications with increased risk of CKD, HT, and hypercalciuria in adulthood, are more evident in those PTNs who were born SGA as a consequence of intrauterine growth restriction secondary to placental insufficiency.

ARTERIAL HT

There is an inverse relationship between birth weight and systolic HT in adolescence (32, 33). A study by Mhanna et al. evaluated blood pressure, obesity, and weight gain as risk factors for HT in 204 patients over 3 years of age, who had been born weighing <1,000 g, with GA of 26 weeks (34). In this population, they found a prevalence of HT of 7.3%, associated with an increase in the body mass index (BMI) and with higher weight gain from birth.

Along the same lines, in another study, over 6,269 PTNs, 528 were SGA and had a higher risk of HT, with the incidence being higher with smaller fetal size. When compared to PTN with adequate birth weight for GA, the SGA had an increased risk of HT of 54% (35).

The risk of presenting HT is also maintained in adulthood. A meta-analysis including preterm-born adults concluded that the mean difference between preterm-born adults and controls was 4.2 mmHg for SBP and 2.6 mmHg for DBP. In another meta-analysis of 1,571 adults born with VLBW (< 1,500 g) vs. 777 full-term controls, mean blood pressure averages were higher for subjects <1,500 g; they had 3.4 mmHg higher SBP and 2.1 mmHg higher DBP than controls. The only perinatal event associated with higher blood pressure was maternal preeclampsia (36). These differences are considerable given that, at the population level, it is estimated that a 2 mmHg reduction in SBP results in a 7 to 14% reduction in mortality from ischemic heart disease and a 9 to 19% reduction in mortality from stroke (35–37).

In PTN and SGA patients, a history of breastfeeding was a protective factor for the development of arterial HT; subjects with a birth weight under 2,500 g who were breastfed had a lower prevalence of HT (38). Both breastfeeding during the first months of life and avoiding rapid weight gain in childhood have been shown to prevent the later risk of obesity and dyslipidemia and reduce glucose tolerance (7).

CHRONIC KIDNEY DISEASE

Reduced nephron endowment and neonatal AKI contribute to the development, HT, and kidney disease (39–42).

Renal function was compared in adolescents born with a history of SGA and mean GA of 27.8 weeks and mean weight of 1,048 g, with adolescents of the same age born at term and birth weight adequate for GA. Whereas there were no differences in blood urea or creatinine values, preterm-born adolescents had a significantly lower GFR compared with term neonates (126.2 vs. 134.3 mL/min/1.73 m^2). Microalbuminuria was found in 7% of PTN patients, especially in women or in those with a high BMI (40). A meta-analysis, which included more than 2 million individuals, found that a history of SGA was associated with an 80% increased likelihood of microalbuminuria. Another study described a 6.3% increase in the urine albumin–creatinine ratio for every 100 g reduction in birth weight (41, 43).

In adults, the incidence of CKD under 43 years of age, who were born PTN, was evaluated in a large cohort study in Sweden. Of the 4,305 participants, 0.1% had a diagnosis of CKD with the overall incidence rate being 4.95 per 100,000 person-years at all ages examined. The incidences per GA at birth were 9.24 for PTN, 5.90 for early term neonates (37–38 weeks), and 4.47 for full-term neonates (39–41 weeks); PTN and early term neonates had two times the risk of CKD compared with full-term neonates. Moreover, GA was inversely related to CKD risk, with the risk being higher in PTNs and SGA; this association was stronger for the development of CKD in childhood and was maintained in adulthood (44). We can hypothesize that the possibility of CKD will be higher in the extremely preterm neonate (<28 weeks) and very preterm (28–32 weeks) compared with moderate to late preterm (32–37 weeks) since they are born in the period of exponential nephrogenesis and exposed to several risk factors that can compromise its correct development.

A Norwegian birth registry study showed that birth weight less than the 10th percentile for the population was associated with a relative risk of 1.7 for end-stage kidney disease (ESKD) during the first 38 years of life, where LBW was associated with an increased risk of ESKD due to any cause (congenital malformations, hereditary diseases, and glomerular diseases) (45, 46). An investigation in a subgroup aged 18–42 years, excluding subjects with congenital renal disease, found that LBW *per se* was not significantly associated with developing ESKD, but being SGA was. In this Norwegian study among those 18–42 years old, being SGA (birth weight less than 10th percentile for GA) was significantly associated with the risk of ESKD, and the effect was much stronger in those born preterm with SGA than those born at term with SGA (RRs of ESKD of 4.02 and 1.41, respectively). These population level data suggest that both SGA and prematurity are important risk factors and likely potentiate each other's effects, with preterm SGA infants being at the highest risk. (45, 47) On the other hand, renal risk in children born preterm was similar between appropriate GA and SGA and also between VLBW and LBW (25).

EVALUATION, DIAGNOSIS, AND PREVENTION

There are currently no guidelines to identify infants at increased risk of developing CKD due to a low number of nephrons, either congenital or acquired.

However, children and adults who were PTN or SGA need long-term follow-up and early preventive actions to help preserve

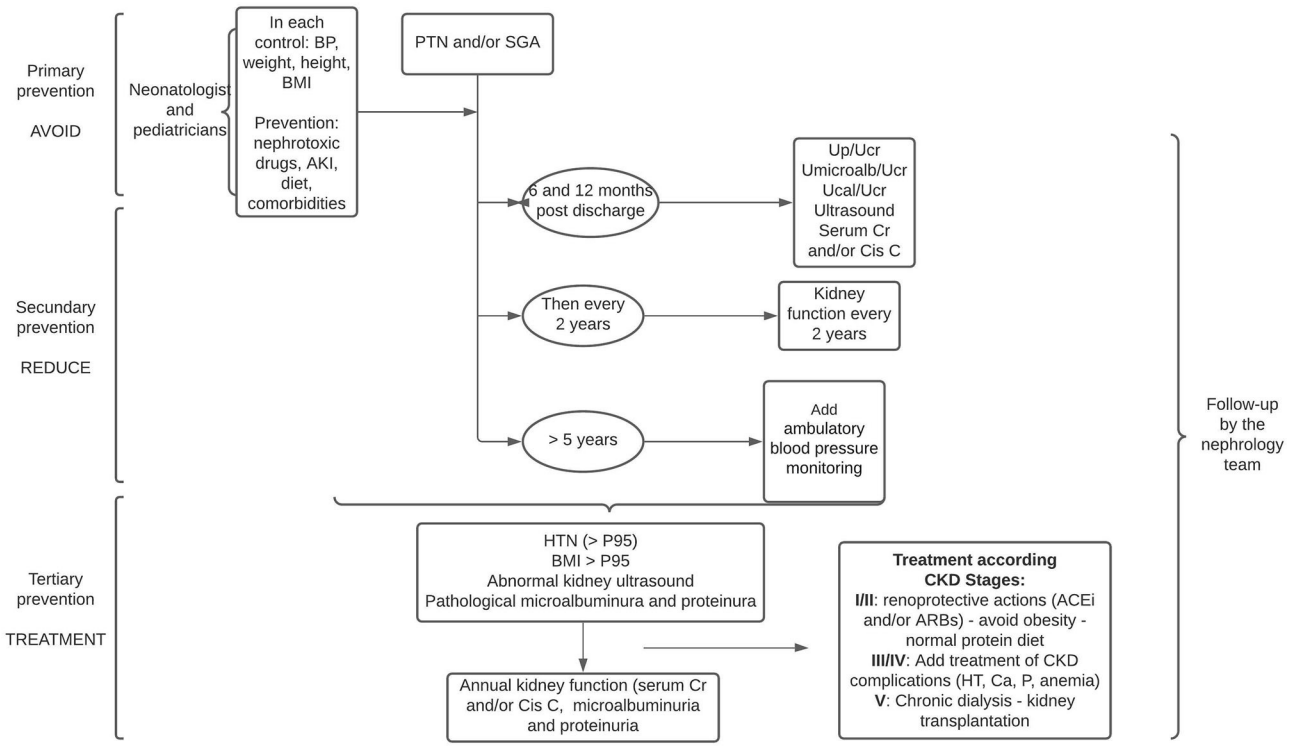


FIGURE 3 | Follow-up proposal for PTN and/or SGA infants in three stages: avoid, reduce, and treat. Cr, creatinine; Cis C, cistatine C; Up/Ucr, urine protein or urine creatinine; UMicroalb/Ucr, urine microalbuminuria or urine creatinine; Ucal/Ucr, urine calcium or urine creatinine; BP, blood pressure; ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers.

renal function and CKD. Clinical follow-up should be structured according to greater or lesser risk of developing CKD in the future with the participation of pediatricians and pediatric nephrologists with varying degrees of intervention (48). On the other hand, obstetricians should monitor fetal development, avoiding all risk factors for prematurity and SGA, in close contact with neonatologists (**Figure 1**).

These interventions should include counseling the parents and then the older patient on how to avoid potentially nephrotoxic drugs exposures (antiinflammatory, antibiotics) (49, 50), other aggravating factors (such as dehydration and urinary tract infection), and control of risk factors for CKD progression (obesity, HT, diabetes, dyslipidemia, anemia, and smoking). HT is an important risk factor for the development of CKD, and effective blood pressure control has been shown to delay the progression of CKD (49). Another risk factor is AKI during the perinatal period with a prevalence between 12.5 and 39.8% in PTN <1,500 g (51), and with progression to subsequent CKD between 10 and 50% (52).

Early detection of potential indicators of hyperfiltration, such as impaired renal reserve, blunted solute clearance, and microalbuminuria, may provide subtle clues to the presence of reduced nephron number (25).

One follow-up option proposed is as follows in all visits for BP controls, assess growth parameters including BMI, and perform family education on the potential risk of CKD, and continue this follow-up until after adolescence and adulthood (49). BP control should begin before 1 year of age (48) and in children over 5 years of age, control with annual ambulatory blood pressure monitoring should be performed (5, 48).

At 6 months after discharge from the neonatal intensive care unit, it is suggested that laboratory tests with serum creatinine and/or cystatin C, and microalbuminuria be performed, and then the periodicity of these tests should be adjusted according to these results or the appearance of comorbidities: history of AKI in the neonatal period or during infancy, HT, obesity, and ultrasound abnormalities. In these cases, blood and urine laboratory controls should be performed annually (48, 49) (**Figure 3**).

The development of nephrocalcinosis in PTNs confers an additional risk for CKD. Nephrocalcinosis in PTN <32 weeks and birth weight <1,500 g has a reported prevalence of 7–64% (53), with a resolution of up to 75% within the first year of life (48, 53).

Although some studies describe alterations in renal size on ultrasound monitoring (small kidneys in preterm patients), there is no evidence to indicate systematic ultrasound monitoring. However, baseline ultrasound is recommended to detect small kidneys, renal asymmetries, or structural alterations.

From the age of 18 years, BP, BMI, serum creatinine, and microalbuminuria should be monitored two times a year until the age of 40 years and then annually (7, 48, 49).

Regarding nutritional recovery, rapid growth (catchup) should be avoided to prevent exacerbation of the renal and cardiovascular risk associated with obesity (44, 45). From childhood onward, an adequate "nephroprotective" dietary pattern should be followed, consisting of a reduction in sodium, carbohydrates, saturated fats, and avoidance of excess protein, combined with increased physical activity and restraint of smoking.

Postnatal catchup growth is encouraged in PTNs and SGA in developing countries with the aim of improving resistance to infections, reducing stunting, malnutrition, and reaching normal neurodevelopment. However, this rapid growth can be linear, or present with unbalanced growth in weight and height, with risk of obesity and HT in adulthood (54, 55). Thus, the rapid and continuous upward crossover of weight percentiles during early childhood, with increasing BMI, has been associated with an increased risk of obesity, HT in adulthood, and progression to CKD in PTN, being more accelerated in those who develop obesity (50). HT should be treated aggressively, and in case of microalbuminuria and/or proteinuria, inhibitors of the renin-angiotensin axis should be indicated.

The importance of this very close follow-up will be to implement treatment in the early stages of CKD (1 o 2, cl $>$ 60 ml/min/1.73 m^2).

The role of strategies played in the clinical management of neonatal intensive therapies in the development of CKD is largely unexplored. Patients are often exposed to medications or situations that compromise nephrogenesis and frequently experience AKI. In recent years, results from the Assessment of Worldwide Acute Kidney Epidemiology in Neonates (AWAKEN) cohort studies have shown the importance of prevention and early detection of AKI given its association with long-term problems (56). If these are independent risk factors for CKD, avoiding nephrotoxins and decreasing the incidence of AKI could lead to better long-term outcomes.

Finally, we must remember that PT and LBW are important risk factors for mortality in childhood and young adulthood (57, 58).

CONCLUSION

Hypertension and CKD have a significant impact on overall morbidity and mortality. It is difficult to quantify the impact of fetal programming on these diseases, but both PTN and SGA have been associated with an alteration in nephrogenesis with the consequent decrease in nephrons, so they have a higher risk of CKD in adulthood, with a higher risk at the lower birth weight (up to 70%). We consider that we are facing a "silent epidemic" of CKD in these patients, so preventive strategies should be implemented early to avoid the progression of CKD. This requires not only a multidisciplinary team (obstetricians, neonatologists, pediatricians, nephrologists, neurologists, cardiologists, and nutritionists), but also public and state measures aimed at awareness, information, and prevention. There are gaps that require collaborative, prospective, and randomized research studies in the area, which will help to optimize cost-effective strategies.

AUTHOR CONTRIBUTIONS

MG and JF: concept and design and drafting of the manuscript. MG, JF, and GM: acquisitions, analysis, and interpretation of data. GM: critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.769734/full#supplementary-material

REFERENCES

1. Alda E, Sebastiani M, Otaño L, Apas A, Corimayo L, Mariani G. Recomendaciones para elmanejo del embarazo y el recién nacido en los límites de la viabilidad. Área de Neonatología. Dirección nacional de maternidad e infancia. Ministerio de Salud, Presidenciade la Nación Argentina. Edición (2014). Available online at: http://www.ms.gba.gov.ar/sitios/tocoginecologia/files/2014/08/Recomendaciones-VIABILIDAD.pdf (accessed July 31, 2021)
2. Mactier H, Bates SE, Johnston T, Lee-Davey C, Marlow N, Mulley K, et al. Perinatal management of extreme preterm birth before 27 weeks of gestation: a framework for practice. Arch Dis Child Fetal Neonatal Ed. (2020) 105:232–39. doi: 10.1136/archdischild-2019-318402
3. Rysavy MA, Horbar JD, Bell EF, Li L, Greenberg LT, Tyson JE, et al. Assessment of an updated neonatal research network extremely preterm birth outcome model in the vermont oxford network. *JAMA Pediatrics*. (2019) 174:e196294. doi: 10.1001/jamapediatrics.2019.6294
4. Síntesis estadística Nro 6 DEIS. Natalidad y Mortalidad. Ministerio de Salud Argentina. (2018) Available online at: https://www.argentina.gob.ar/salud/deis (accessed August 07, 2021)
5. Solís, A, Cerda, J, González, C. Ambulatory blood pressure monitoring in school children with a history of extreme prematurity. *Rev Chil Pediatr*. (2018) 89:18–23. doi: 10.4067/S0370-41062018000100018
6. Sutherland MR, Bertagnolli M, Lukaszewski MA, Huyard F, Yzydorczyk C, et al. Preterm birth and hypertension risk: the oxidative stress paradigm. *Hypertension (Dallas, Tex.: 1979)*. (2014) 63:12–18. doi: 10.1161/HYPERTENSIONAHA.113.01276
7. Low Birth Weight and Nephron Number Working Group. The impact of kidney development on the life course: a consensus document for action. *Nephron*. (2017) 136:3–49. doi: 10.1159/000457967
8. Herrera-Garcia G, Contag S. Maternal preeclampsia and risk for cardiovascular disease in offspring. *Curr Hypertens Rep*. (2014) 16:475. doi: 10.1007/s11906-014-0475-3
9. Stritzke A, Thomas S, Amin H, Fusch C, Lodha A. Renal consequences of preterm birth. *Mol Cellul Pediat*. (2017) 4:2. doi: 10.1186/s40348-016-0068-0
10. Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. *Reproduction (Cambridge, England)*. (2009) 138:895–902. doi: 10.1530/REP-09-0092
11. Crump C, Sundquist K, Sundquist J, Winkleby MA. Gestational age at birth and mortality in young adulthood. *JAMA*. (2011) 306:1233–40. doi: 10.1001/jama.2011.1331
12. Black MJ, Sutherland MR, Gubhaju L, Kent AL, Dahlstrom JE, Moore L. When birth comes early: effects on nephrogenesis.

Nephrology (Carlton, Vic.). (2013) 18:180–2. doi: 10.1111/nep.12028
13. Hoy WE, Hughson MD, Bertram JF, Douglas-Denton R, Amann K. Nephron number, hypertension, renal disease, and renal failure. *J Am Soc Nephrol.* (2005) 16:2557–64. doi: 10.1681/ASN.2005020172
14. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF, et al. A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy. *Kidney Int Suppl.* (2003) S31–7. doi: 10.1046/j.1523-1755.63.s83.8.x
15. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol. (1992) 99:296–301. doi: 10.1111/j.1471-0528.1992.tb13726.x
16. Langley-Evans SC, Welham SJ, Jackson AA. Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci.* (1999) 64:965–74. doi: 10.1016/S0024-3205(99)00022-3
17. Brenner BM, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int.* (1996) 49:1774–7. doi: 10.1038/ki.1996.265
18. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol.* (1981) 241:F85–93. doi: 10.1152/ajprenal.1981.241.1.F85
19. Matos P, Duarte-Silva M, Drukker A, Guignard JP. Creatinine reabsorption by the newborn rabbit kidney. *Pediatric Res.* (1998) 639–41. doi: 10.1203/00006450-199811000-00004
20. Chevalier RL. Evolutionary nephrology. international society of nephrology. (2017). Charlottesville: Elsevier Inc. doi: 10.1016/j.ekir.2017.01.012
21. Cheong JN, Wlodek ME, Moritz KM, Cuffe JS. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations. *J Physiol.* (2016) 594:4727–40. doi: 10.1113/JP271745
22. Gubhaju L, Sutherland MR, Black MJ. Preterm birth and the kidney: implications for long-term renal health. *Reproduct Sci. (Thousand Oaks, Calif.)*. (2011) 18:322–33. doi: 10.1177/1933719111401659
23. Rodríguez MM, Gómez AH, Abitbol CL, Chandar JJ, Duara S, Zilleruelo GE. Histomorphometric analysis of postnatal glomerulogenesis in extremely preterm infants. *Pediatr Dev Pathol.* (2004) 7:17–25. doi: 10.1007/s10024-003-3029-2
24. Chevalier RL, Forbes MS, Thornhill BA. Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. *Kidney Int.* (2009) 75:1145–52. doi: 10.1038/ki.2009.86
25. Puddu M, Fanos V, Podda F, Zaffanello M. The kidney from prenatal to adult life: perinatal programming and reduction of number of nephrons during development. *Am J Nephrol.* (2009) 30:162–70. doi: 10.1159/000211324
26. Gallini F, Maggio L, Romagnoli C, Marrocco G, Tortorolo G. Progression of renal function in preterm neonates with gestational age < or = 32 weeks. *Pediatric Nephrol. (Berlin, Germany)*. (2000) 15:119–24. doi: 10.1007/s004670000356
27. Carmody JB, Swanson JR, Rhone ET, Charlton JR. Recognition and reporting of AKI in very low birth weight infants. *Clin J Am Soc Nephrol.* (2014) 9:2036–43. doi: 10.2215/CJN.05190514
28. Andersen LG, Angquist L, Eriksson JG, Forsen T, Gamborg M, Osmond C, et al. Birth weight, childhood body mass index and risk of coronary heart disease in adults: combined historical cohort studies. *PLoS ONE.* (2010) 5:e14126. doi: 10.1371/journal.pone.0014126
29. Rodríguez LI, Miceli SC, D'Urso Villar M, Maurizi M, Caram ME, Pérez MZ, et al. Tamaño renal, presión arterial, microalbuminuria y filtración glomerular son aconsejables controlar en el seguimiento de niños nacidos prematuros. *Revista De Nefrología, Diálisis Y Trasplante.* (2021) 41:9–22.
30. Pérez González E, Marín Serra J. Evaluación de la función renal en el recién nacido. Protoc diagn ter pediatr. (2014) 1:3 7–51. Asociación Española de Pediatría. Available online at: https://www.aeped.es/protocolos/
31. Comité Nacional de. Nefrología, Sociedad Argentina de Pediatría. *El riñón del recién ncido Nefrología pediátrica 3era edición Buenos Aires*. (2017).
32. Hirschler V, Bugna J, Roque M, Gilligan T, Gonzalez C. Does low birth weight predict obesity/overweight and metabolic syndrome in elementary school children?. *Archives of Medical Res.* (2008) 39:796–802. doi: 10.1016/j.arcmed.2008.08.003
33. Yuan ZP, Yang M, Liang L, Fu JF, Xiong F, Liu GL, et al. Possible role of birth weight on general and central obesity in Chinese children and adolescents: a cross-sectional study. *Ann Epidemiol.* (2015) 25:748–52. doi: 10.1016/j.annepidem.2015.05.011
34. Mhanna MJ, Iqbal AM, Kaelber DC. Weight gain and hypertension at three years of age and older in extremely low birth weight infants. *J Neonatal-Perinatal Med.* (2015) 8:363–9. doi: 10.3233/NPM-15814080
35. Bonamy AK, Norman M, Kaijser M. Being born too small, too early, or both: does it matter for risk of hypertension in the elderly? *Am J Hypertens.*. (2008) 21:1107–10. doi: 10.1038/ajh.2008.241
36. Hovi P, Vohr B, Ment LR, Doyle LW, McGarvey L, Morrison KM, et al. Blood pressure in young adults born at very low birth weight: adults born preterm international collaboration. *Hypertension (Dallas, Tex.:1979)*. (2016) 68:880–7. doi: 10.1161/HYPERTENSIONAHA.116.08167
37. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, et al. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet (London, England)*. (2002) 360:1903–13. doi: 10.1016/S0140-6736(02)11911-8
38. Zarrati M, Shidfar F, Razmpoosh E, Nezhad FN, Keivani H, Hemami MR, et al. Does low birth weight predict hypertension and obesity in school children? *Ann Nutrit Metabolism*. (2013) 63:69–76. doi: 10.1159/000351869
39. Carmody JB, Charlton JR. Short-term gestation, long-term risk: prematurity and chronic kidney disease. *Pediatrics.* (2013) 131:1168–79. doi: 10.1542/peds.2013-0009
40. South AM, Nixon PA, Chappell MC, Diz DI, Russell GB, Jensen ET, et al. Renal function and blood pressure are altered in adolescents born preterm. *Pediatric Nephrol. (Berlin, Germany)*. (2019) 34:137–44. doi: 10.1007/s00467-018-4050-z
41. Koleganova N, Piecha G, Ritz E. Prenatal causes of kidney disease. Blood Purif. (2009) 27:48–52. doi: 10.1159/000167008
42. Hoy WE, Rees M, Kile E, Mathews JD, Wang Z. A new dimension to the Barker hypothesis: low birthweight and susceptibility to renal disease. *Kidney Int.* (1999) 56:1072–7. doi: 10.1046/j.1523-1755.1999.00633.x
43. White SL, Perkovic V, Cass A, Chang CL, Poulter NR, Spector T, et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. *Am J Kidney Dis.* (2009) 54:248–61. doi: 10.1053/j.ajkd.2008.12.042
44. Crump C, Sundquist J, Winkleby MA, Sundquist K. Preterm birth and risk of chronic kidney disease from childhood into mid-adulthood: national cohort study. *BMJ (Clinical research ed)*. (2019) 365:1346. doi: 10.1136/bmj.l1346
45. Luyckx VA, Brenner BM. Birth weight, malnutrition and kidney-associated outcomes–a global concern. Nature reviews. *Nephrology.* (2015) 11:135–49. doi: 10.1038/nrneph.2014.251
46. Vikse BE, Irgens LM, Leivestad T, Hallan S, Iversen BM. Low birth weight increases risk for end-stage renal disease. *J Am Soc Nephrol.* (2008) 19:151–7. doi: 10.1681/ASN.2007020252
47. Ruggajo P, Skrunes R, Svarstad E, Skjærven R, Reisæther AV, Vikse BE. Familial factors, low birth weight, and development of ESRD: A nationwide registry study. *Am J Kidney Dis.* (2016) 67:601–8. doi: 10.1053/j.ajkd.2015.11.015
48. Cavagnaro Felipe. El riñón del niño prematuro: Riesgos a largo plazo. Revista Chilena de Pediatría. (2020) 91:324. doi: 10.32641/rchped.v91i3.1607
49. Luyckx VA, Perico N, Somaschini M, Manfellotto D, Valensise H, et al. A developmental approach to the prevention of hypertension and kidney disease: a report from the Low Birth Weight and Nephron Number Working Group. *Lancet (London, England)*. (2017) 390:424–8. doi: 10.1016/S0140-6736(17)30576-7
50. Luyckx VA. Preterm Birth and its Impact on Renal Health. *Seminars Nephrol.* (2017) 37:311–9. doi: 10.1016/j.semnephrol.2017.05.002
51. Chaturvedi S, Ng KH, Mammen C. The path to chronic kidney disease following acute kidney injury: a neonatal perspective. *Pediatric Nephrol. (Berlin, Germany)*. (2017) 32:227–41. doi: 10.1007/s00467-015-3298-9
52. Selewski DT, Charlton JR, Jetton JG, Guillet R, Mhanna MJ, Askenazi DJ, et al. Neonatal Acute Kidney Injury. *Pediatrics.* (2015) 136:e463–73. doi: 10.1542/peds.2014-3819
53. Schell-Feith, E. A., Kist-van Holthe, J. E., & van der Heijden, A. J. (2010). Nephrocalcinosis in preterm neonates. *Pediatric Nephrol. (Berlin, Germany)*. 25:221–30. doi: 10.1007/s00467-008-0908-9

54. Goldberg DL, Becker PJ, Brigham K, Carlson S, Fleck L, Gollins L, et al. Identifying malnutrition in preterm and neonatal populations: recommended indicators. *J Academy Nutr Dietet.* (2018) 118:1571–82. doi: 10.1016/j.jand.2017.10.006
55. Dogra S, Thakur A, Garg P, Kler N. Effect of differential enteral protein on growth and neurodevelopment in infants <1500 g: A randomized controlled trial. *J Pediat Gastroenterol Nutr.* (2017) 64:e126–32. doi: 10.1097/MPG.0000000000001451
56. Askenazi DJ. AWAKEN-Ing a new frontier in neonatal nephrology. *Front Pediatr.* (2020) 8:21. doi: 10.3389/fped.2020.00021
57. Risnes KR, Vatten LJ, Baker JL, Jameson K, Sovio U, Kajantie E, et al. Birthweight and mortality in adulthood: a systematic review and meta-analysis. *Int J Epidemiol.* (2011) 40:647–61. doi: 10.1093/ije/dyq267
58. Bates ML, Levy PT, Nuyt AM, Goss KN, Lewandowski AJ, McNamara PJ. Adult cardiovascular health risk and cardiovascular phenotypes of prematurity. *J Pediatr.* (2020) 227:17–30. doi: 10.1016/j.jpeds.2020.09.019

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

ORIGINAL RESEARCH
published: 21 June 2022
doi: 10.3389/fmed.2022.863275

SLC12A3 Variation and Renal Function in Chinese Patients With Hypertension

Chin-Chou Huang[1,2,3,4*], *Chia-Min Chung*[5,6], *Chih-Yu Yang*[2,7,8], *Hsin-Bang Leu*[1,2,4,9], *Po-Hsun Huang*[1,4,7,10], *Liang-Yu Lin*[2,4,11], *Tao-Cheng Wu*[1,2,4], *Shing-Jong Lin*[1,4,7,12], *Wen-Harn Pan*[13,14] and *Jaw-Wen Chen*[1,3,4,9]

[1] Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [2] School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [3] Institute of Pharmacology, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [4] Cardiovascular Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan, [5] Center for Drug Abuse and Addiction, China Medical University Hospital, Taichung, Taiwan, [6] Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, [7] Institute of Clinical Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, [8] Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [9] Healthcare and Services Center, Taipei Veterans General Hospital, Taipei, Taiwan, [10] Department of Critical Care Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [11] Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, [12] Taipei Heart Institute, Taipei Medical University, Taipei, Taiwan, [13] Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [14] Institute of Epidemiology, School of Public Health, National Taiwan University, Taipei, Taiwan

OPEN ACCESS

Edited by:
Carmen Tzanno-Martins, Hospital Alemão Oswaldo Cruz, Brazil

Reviewed by:
Daw-Yang Hwang, National Health Research Institutes, Taiwan
Ingrid Inge Prkacin, University of Zagreb, Croatia

***Correspondence:**
Chin-Chou Huang
cchuang4@vghtpe.gov.tw; huangchinchou@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *27 January 2022*
Accepted: *19 April 2022*
Published: *21 June 2022*

Citation:
Huang C-C, Chung C-M, Yang C-Y, Leu H-B, Huang P-H, Lin L-Y, Wu T-C, Lin S-J, Pan W-H and Chen J-W (2022) SLC12A3 Variation and Renal Function in Chinese Patients With Hypertension. Front. Med. 9:863275. doi: 10.3389/fmed.2022.863275

Objective: *SLC12A3* (solute carrier family 12 member 3) gene variants are associated with diabetic nephropathy; however, their association with hypertensive nephropathy remains unknown. We aimed to investigate the association between *SLC12A3* gene polymorphisms and renal function in patients with hypertension.

Methods: Participants from three non-diabetic hypertensive cohorts, including young-onset hypertension (cohort 1, $n = 882$), treatment-naïve hypertension (cohort 2, $n = 90$), and follow-up cohort (cohort 3, $n = 166$), underwent genotyping for single nucleotide polymorphisms in *SLC12A3*. Renal events were defined as a >25 and >50% decline in estimated glomerular filtration rate (eGFR).

Results: In cohort 1, *SLC12A3* rs16963397 C/C or C/G ($P = 0.005$), rs13334864 C/C or C/T ($P = 0.020$), and rs7187932 A/A or A/G polymorphisms ($P = 0.014$) had higher eGFRs compared to their counterparts, with similar findings observed in cohort 2. In cohort 3, over a mean follow-up of 5.8 ± 1.7 years, participants with either *SLC12A3* rs16963397 C/C or rs13334864 C/C polymorphisms had more >25 and >50% eGFR decline than their counterparts (log-rank test, $P = 0.058$ and $P = 0.038$, respectively). Cox regression analysis revealed that *SLC12A3* rs16963397 C/C and rs13334864 C/C polymorphisms were significantly associated with an increased risk of >25% [hazard ratio (HR), 3.294; 95% confidence interval (CI), 1.158–9.368; $P = 0.025$] and >50% decline in eGFR (HR, 18.630; 95% CI, 1.529–227.005, $P = 0.022$) than their counterparts.

Conclusion: *SLC12A3* polymorphisms are associated with renal function in Chinese patients with hypertension.

Keywords: glomerular filtration rate, glomerular hyperfiltration, hypertension, hypertensive nephropathy, SLC12A3

INTRODUCTION

Hypertension is an important public health challenge worldwide (1, 2). More than one-fourth of the global adult population has hypertension, and its incidence is rising (3). Hypertension is one of the most important factors for chronic kidney disease (CKD) and end stage renal disease (ESRD), and the importance of hypertension management in renal protection cannot be overemphasized (4, 5).

The *SLC12A3* (solute carrier family 12 member 3) gene is located on chromosome 16 (16q13). *SLC12A3* encodes the thiazide-sensitive Na-Cl cotransporter (NCC) in the luminal membrane of the distal convoluted tubule (6, 7). Mutations in NCC have been reported to be responsible for Gitelman's syndrome (8, 9), an autosomal recessive renal tubular disorder characterized by hypokalemia, hypomagnesemia, hypocalciuria, and metabolic alkalosis. It has also been reported that *SLC12A3* polymorphism is linked to the effects of thiazide diuretics (10).

Several studies have reported a link between *SLC12A3* gene variants and diabetic nephropathy (11–18). However, the association between genetic variations in *SLC12A3* and hypertensive nephropathy remains unknown. Therefore, we aimed to investigate the role of *SLC12A3* gene polymorphisms on renal function in non-diabetic hypertensive patients in a Chinese population.

MATERIALS AND METHODS

Study Population

The study involved three non-diabetic hypertensive cohorts, including a young-onset hypertension (YOH) cohort (cohort 1), a treatment-naïve cohort (cohort 2), and a follow-up cohort (cohort 3) (**Figure 1**).

In cohort 1, non-diabetic participants with YOH were recruited from six medical centers in Taiwan between 2004 and 2005. The inclusion criteria were as follows: age 20–50 years; subjects who met one of the following criteria: (i) subject's systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$90 mmHg in at least two consecutive visiting 2 months, (ii) subject taking one anti-hypertensive medication in 2 months; body mass index (BMI) $\leq$ 35 kg/m^2; fasting glucose level $<$ 126 mg/dL with no diabetes mellitus; no medical history of severe diseases; and no acute disease in the 2 weeks prior to the visit. Participants with secondary hypertension were excluded from the study (19).

In cohort 2, participants with newly diagnosed hypertension who were previously untreated were prospectively included in the study if all of the following criteria were fulfilled: age $\geq$ 25 years; a sitting office SBP of 140–180 mmHg and/or a DBP of 90–110 mmHg on three different occasions within 3 months before the study, fasting plasma sugar level <126 mg/dL with no diabetes mellitus, and no clinical evidence of secondary hypertension. Participants with the following characteristics were excluded: current use of antihypertensive drugs; history of major systemic disease in the 3 months prior to the study; renal dysfunction with a plasma creatinine level of >1.7 mg/dL; liver dysfunction with liver enzyme activity of more than double the normal upper limit; congestive heart failure with New York Heart Association function class II-IV; and pregnancy.

In cohort 3, participants diagnosed with hypertension from February 2012 to December 2020 at the Taipei Veteran General Hospital were included. The inclusion criteria were as follows: age $\geq$ 20 years; essential hypertensive patients; no medical history of severe diseases; and no acute disease in the 2 weeks prior to the study. Participants with secondary hypertension were excluded from the study (20). Only participants without diabetes mellitus were included in the present study.

The study protocols were approved by the Ethics Committee of the Academic Sinica and Taipei Veterans General Hospital. All participants agreed to participate after being informed of the nature and purpose of the study. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Study Design

A flow chart of the study is presented in **Figure 1**. This study was conducted using three non-diabetic hypertensive cohorts. In cohorts 1 and 2, two cross-sectional cohorts, the association between *SLC12A3* polymorphisms and renal function was investigated in participants with YOH (cohort 1) and treatment-naïve hypertension (cohort 2). In cohort 3, a longitudinal cohort, the potential impact of *SLC12A3* polymorphisms on renal function decline was investigated in participants with essential hypertension. Renal function was assessed every 3 months.

Prescribed antihypertensive agents were recorded, including angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), beta-blockers, calcium channel blockers (CCBs), and thiazide diuretics. All participants underwent genotyping for single nucleotide polymorphisms (SNPs) in the *SLC12A3* gene.

Blood Pressure Measurements

Office BP was measured according to a standardized protocol by a well-trained nurse with an electronic BP monitor (cohort 1, Omega 1400 NBP, Invivo Research Inc., Orlando, FL, USA; cohorts 2 and 3, Omron HEM-7121, Omron Healthcare Taiwan Co., Songshan, Taipei, Taiwan, ROC) in the morning after participants had been instructed to sit for 10 min in a quiet room. Three consecutive BP measurements were obtained from the same upper arm, with each measurement taken at 30-s intervals.

For ambulatory BP monitoring (ABPM), participants were connected to an ABPM device between 08:00 and 10:00 h. The device was programmed to record BP every 15 min between 06:00 and 22:00 h (daytime BP), and every 30 min from 22:00 to 0600 h (nighttime BP).

Laboratory Measurements

Blood samples were collected in the morning after overnight fasting. All blood samples were sent to the central laboratory for analysis. The participants were instructed to take all routine medications, as they normally would. The blood samples were centrifuged, and the serum was thawed for analysis. The estimated glomerular filtration rate (eGFR) was calculated using the four-variable equation proposed by the Modification of Diet in Renal Disease Study (21).

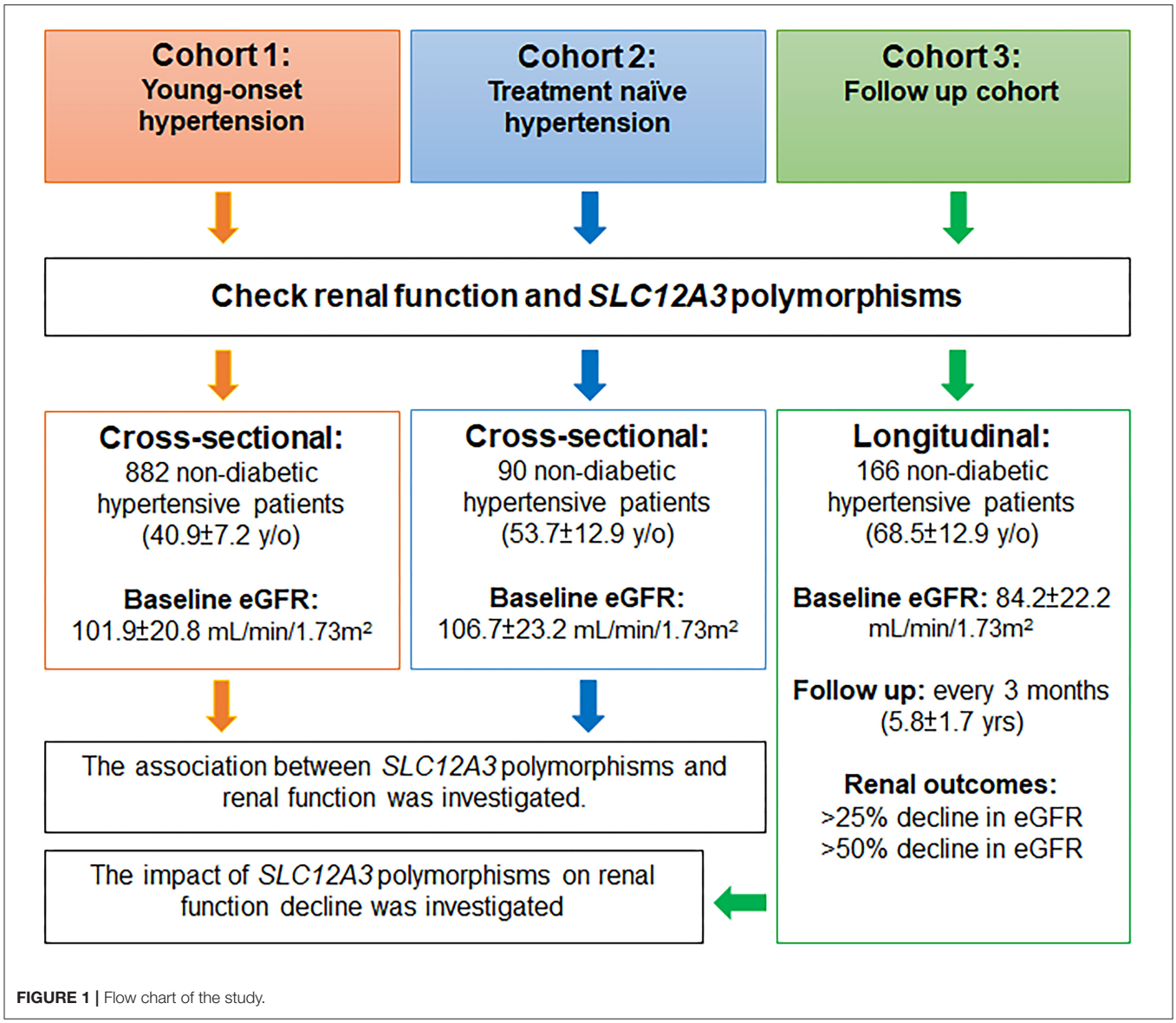


FIGURE 1 | Flow chart of the study.

Twenty-Four-Hour Urine Collection

In cohort 1, every participant was provided with a urine sample container. Urine samples were collected over a 24-h period by a well-trained nurse. Complete oral and written guidance about urine specimen collection, transportation, and preservation were also provided. On the day of urine collection, the participants were required to follow their daily diet habits, and they were advised to avoid strenuous exercise in order to reduce sweating. The participants were required to discard the first voided urine upon waking up in the morning and to collect all voided urine during the subsequent 24 h, including the first void sample of the following morning. Upon completion of collection, a well-trained nurse recorded the urine volume in each collection container to determine the total urine volume during the 24-h collection period. All urine samples were sent to a central laboratory to determine the levels of urinary sodium, potassium, chloride, and creatinine.

Selection of Candidate Gene

The *SLC12A3* gene was evaluated in this study. We searched for SNPs in the gene using the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/) and selected SNPs with a minor allele frequency >0.05 as the genotyping markers. We investigated six tag SNP markers in the introns of the *SLC12A3* gene, including rs16963397, rs13334864, rs7187932, rs12449275, rs12447287, and rs1138429.

Genotyping

A total of 20 cc of blood was collected from each participant. Genomic DNA was isolated from peripheral lymphocytes using

TABLE 1 | Baseline characteristics of the participants.

	Cohort 1	Cohort 2	Cohort 3
Patient number, *n*	882	90	166
Age, years	40.9 ± 7.2	53.7 ± 12.9	68.5 ± 12.9
Male, *n* (%)	609 (69.0%)	45 (50.0%)	99 (59.6%)
BMI, kg/m^2	26.5 ± 3.5	25.8 ± 3.8	25.9 ± 3.8
Office SBP, mmHg	129.1 ± 14.6	143.6 ± 17.2	130.5 ± 17.3
Office DBP, mmHg	86.2 ± 11.6	93.0 ± 12.3	80.0 ± 11.1
ACEI/ARB, *n* (%)	377 (42.7%)	–	102 (61.4%)
Beta-blocker, *n* (%)	398 (45.1%)	–	44 (26.5%)
CCB, *n* (%)	369 (41.8%)	–	115 (69.3%)
Thiazide, *n* (%)	143 (16.2%)	–	57 (34.3%)
eGFR, mL/min/1.73 m^2	101.9 ± 20.8	106.7 ± 23.2	84.2 ± 22.2
Creatinine, mg/dL	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.2
Mean follow up duration, years	–	–	5.8 ± 1.7

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure.

the phenol/chloroform extraction method. SNP genotyping was performed using high-throughput matrix-assisted laser desorption and ionization–time of flight (MALDI-TOF) mass spectrometry. Briefly, primers and probes were designed using SpectroDESIGNER software (Sequenom, San Diego, California, USA). Multiplex PCRs were performed, and unincorporated ddNTPs were dephosphorylated using shrimp alkaline phosphatase (Hoffman-LaRoche, Basel, Switzerland), followed by primer extension. The purified primer extension reaction was spotted onto a 384-element silicon chip (SpectroCHIP, Sequenom) and analyzed using an autoflex MALDI-TOF SpectroREADER mass spectrometer (Sequenom); the resulting spectra were processed with SpectroTYPER (Sequenom). The people who performed the genetic study were blinded to the clinical data of the study subjects.

Renal Outcomes

The participants in cohort 3 were followed up to assess renal function decline. Renal events during the follow-up period were defined as minor nephropathy, >25% decline in eGFR, and major nephropathy, >50% decline in eGFR; these definitions have been used previously (20, 22).

Statistical Analysis

The participant characteristics were summarized using descriptive statistics. Quantitative variables are expressed as the mean ± standard deviation (SD), and categorical variables are expressed as frequencies (percentages). Parametric continuous data between different groups were compared using Student's *t*-test. Non-parametric data between different groups were compared using the Mann–Whitney test. Categorical variables were analyzed using the chi-square test or Fisher's exact test.

In cohort 1, genetic association analyses were conducted using a general linear model to evaluate the relationship between eGFR and additive, dominant, and recessive model assumptions. Statistical significance was determined according to the lowest *P*-value ($P < 0.05$) in the three models. Multivariate analysis was performed using linear regression after adjusting for potential confounding factors, including age, sex, BMI, office BP ≥ 140/90 mmHg, ACEI/ARB, beta-blocker, CCB, thiazide diuretics, and baseline eGFR.

In cohort 3, survival analysis was assessed using the Kaplan–Meier curve, with significance based on the log-rank test. Cox proportional hazard regression analysis was performed to assess the independent effects of *SLC12A3* polymorphism and renal outcomes. The adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated after adjusting for potential confounding factors, including age, sex, BMI, office BP ≥ 140/90 mmHg, ACEI/ARB, beta-blocker, CCB, thiazide diuretics, and baseline eGFR.

Statistical significance was defined as a two-sided $P < 0.05$. Statistical analysis was performed using SPSS software (Version 21.0, SPSS Inc., Chicago, IL, USA).

RESULTS

SLC12A3 Polymorphisms and Renal Function in Patients With YOH: Cohort 1

In cohort 1, 882 non-diabetic participants with YOH were genotyped for *SLC12A3*. The mean age of the participants was 40.9 ± 7.2 years, and ∼69.0% were men. The average BMI of the participants was 26.5 ± 3.5 kg/m^2, the mean SBP was 129.1 ± 14.6 mmHg, and the mean DBP was 86.2 ± 11.6 mmHg. The use of antihypertensive agents included ACEIs/ARBs (42.7%), beta-blockers (45.1%), CCBs (41.8%), and thiazide diuretics (16.2%). The serum creatinine level was 0.8 ± 0.2 mg/dL, and the eGFR was 101.9 ± 20.8 mL/min/1.73 m^2 (**Table 1**, **Figure 1**).

The genetic information regarding the *SLC12A3* polymorphisms in cohort 1 is presented in **Table 2**. Of the six SNPs in *SLC12A3*, rs16963397 ($r = 0.094$, $P = 0.005$), rs13334864 ($r = 0.078$, $P = 0.020$), and rs7187932 ($r = 0.083$, $P = 0.014$) polymorphisms were associated with eGFR (**Table 2**).

Overall, participants with *SLC12A3* rs16963397 C/C or C/G polymorphisms, rs13334864 C/C or C/T polymorphisms, and rs7187932 A/A or A/G polymorphisms had higher eGFR and higher 24-h urine sodium excretion than their counterparts (**Supplementary Tables 1–6**).

Multivariate linear regression revealed that *SLC12A3* rs16963397 C/C or C/G polymorphisms (β = 3.183, 95% CI = 0.547–5.818, $P = 0.018$), *SLC12A3* rs13334864 C/C or C/T polymorphisms (β = 2.784, 95% CI = 0.180–5.388, $P = 0.036$), and *SLC12A3* rs7187932 A/A or A/G polymorphisms (β = 2.778, 95% CI = 0.133–5.423, $P = 0.040$) were independently associated with eGFR (**Table 3**).

SLC12A3 Polymorphisms and Renal Function in Patients With Treatment-Naïve Hypertension: Cohort 2

Cohort 2 comprised 90 non-diabetic participants with treatment-naïve hypertension. The mean age of the participants was 53.7 ± 12.9 years, and ∼50.0% were men. The average BMI of

TABLE 2 | Association of individual SLC12A3 gene variants and estimated glomerular filtration rate (cohort 1).

SNP	Position	Role	Genotype	Frequency	Allele	MAF	HWE	*r*	*P*-value
rs16963397	chr16:56927441	Intron	C/C	55 (6.2%)	G:C	0.244	0.636	0.094	0.005
			C/G	320 (36.3%)					
			G/G	507 (57.5%)					
rs13334864	chr16:56929841	Intron	C/C	131 (14.9%)	T:C	0.406	<0.001	0.078	0.020
			C/T	325 (36.8%)					
			T/T	426 (48.3%)					
rs7187932	chr16:56931704	Intron	A/A	63 (7.1%)	G:A	0.245	0.073	0.083	0.014
			A/G	307 (34.8%)					
			G/G	512 (58.0%)					
rs12449275	chr16:56937855	Intron	A/A	35 (4.0%)	G:A	0.124	<0.001	0.044	0.188
			A/G	148 (16.8%)					
			G/G	699 (79.3%)					
rs12447287	chr16:56937998	Intron	C/C	43 (4.9%)	T:C	0.145	<0.001	0.054	0.109
			C/T	170 (19.3%)					
			T/T	669 (75.9%)					
rs1138429	chr16:56942921	Intron	A/A	626 (71.0%)	A:T	0.162	0.091	0.025	0.461
			A/T	226 (25.6%)					
			T/T	30 (3.4%)					

HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; r, correlation coefficient; SNP, single nucleotide polymorphisms.
Alleles shown are major:minor.

TABLE 3 | Multivariate analysis for estimated glomerular filtration rate (cohort 1).

	Model 1			Model 2		
	β	(95% CI)	*P*-value	β	(95% CI)	*P*-value
rs16963397 (C/C or C/G vs. G/G)	3.071	(0.464–5.679)	0.021	3.183	(0.547–5.818)	0.018
rs13334864 (C/C or C/T vs. T/T)	2.701	(0.119–5.283)	0.040	2.784	(0.180–5.388)	0.036
rs7187932 (A/A or A/G vs. G/G)	2.649	(0.034–5.264)	0.047	2.778	(0.133–5.423)	0.040

β, unstandardized coefficient; CI, confidence interval.
Model 1: Adjusted for age and male.
Model 2: Adjusted for age, male, BMI, office BP ≥ 140/90 mmHg, ACEI/ARB, beta-blocker, CCB, and thiazide.

the participants was 25.8 ± 3.8 kg/m^2, the mean SBP before treatment was 143.6 ± 17.2 mmHg, and the mean DBP before treatment was 93.0 ± 12.3 mmHg. The serum creatinine level was 0.7 ± 0.2 mg/dL and the eGFR was 106.9 ± 23.0 mL/min/1.73 m^2 (**Table 1**, **Figure 1**).

Overall, participants with *SLC12A3* rs16963397 C/C or C/G polymorphisms, rs13334864 C/C or C/T polymorphisms, and rs7187932 A/A or A/G polymorphisms had lower serum creatinine and/or higher eGFR than their counterparts (**Supplementary Tables 7–12**).

SLC12A3 Polymorphisms and Renal Function Decline: Cohort 3

In cohort 3, 166 participants with non-diabetic hypertension were included. The mean age of the participants was 68.5 ± 12.9 years, and approximately 59.6% were men. The average BMI of the participants was 25.9 ± 3.8 kg/m^2, the baseline SBP was 130.5 ± 17.3 mmHg, and the baseline DBP was 80.0 ± 11.1 mmHg. The antihypertensive agents used included ACEIs/ARBs (61.4%), beta-blockers (26.5%), CCBs (69.3%), and thiazide diuretics (34.3%). The renal function of the participants upon enrollment was generally not impaired (serum creatinine level of 0.9 ± 0.2 mg/dL and eGFR of 84.2 ± 22.2 mL/min/1.73 m^2) (**Table 1**, **Figure 1**).

Overall, the baseline renal function, either eGFR or serum creatinine, was similar between participants with different *SLC12A3* polymorphisms (**Supplementary Tables 13–18**).

During an average follow up period of 5.8 ± 1.7 years, >25% decline in eGFR was noted in 40 hypertensive participants and >50% decline in eGFR was noted in 8 hypertensive participants. Survival was assessed using Kaplan–Meier analysis. Participants with *SLC12A3* rs16963397 C/C polymorphism had more >25 and >50% eGFR decline than those with C/G or G/G polymorphisms, respectively (log-rank test, $P = 0.058$ and $P = 0.038$, respectively) (**Figures 2A,B**). Participants with the *SLC12A3* rs13334864 C/C polymorphism had more >25 and >50% eGFR decline than those with C/T or T/T polymorphisms (log-rank test, $P = 0.058$ and $P = 0.038$, respectively). The

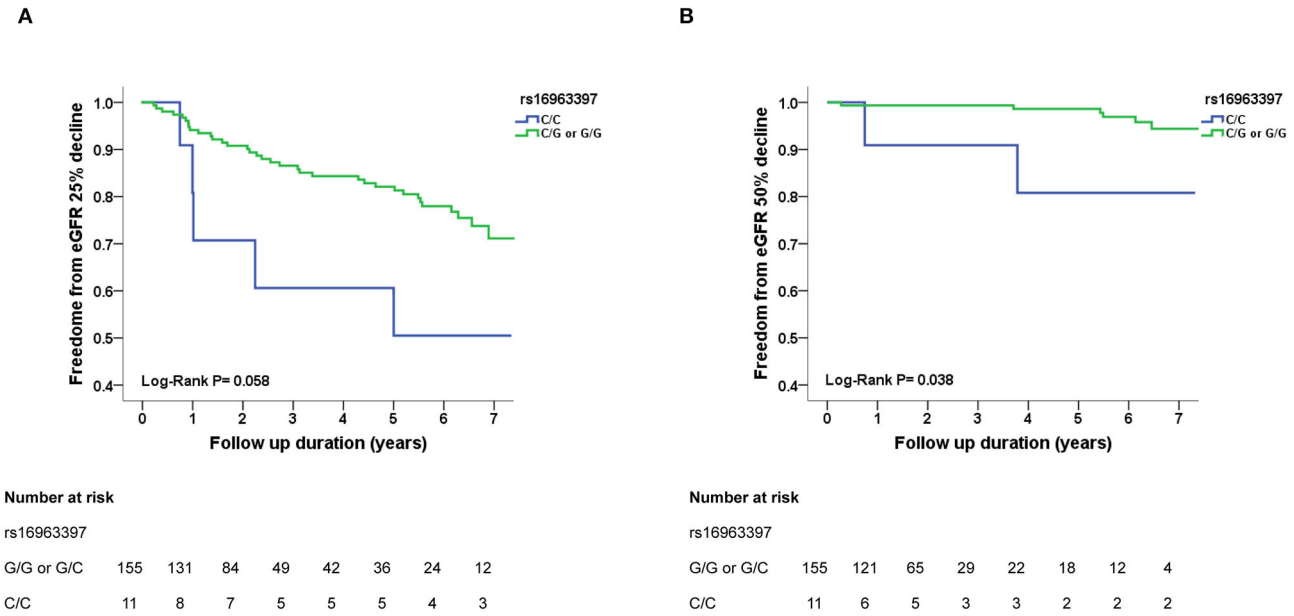


FIGURE 2 | Kaplan–Meier survival curves showing the absence of renal events with respect to *SLC12A3* rs16963397 polymorphisms in patients with hypertension in cohort 3. All participants were divided into two groups according to *SLC12A3* rs16963397 polymorphisms. In panel A and B, the blue line represents the patient group with *SLC12A3* rs16963397 C/C polymorphisms, and the green line represents the group with *SLC12A3* rs16963397 C/G or G/G polymorphisms. Renal events were defined as >25% decline and >50% decline in estimated glomerular filtration rate (eGFR). Differences were compared using the log-rank test. **(A)** *SLC12A3* rs16963397 polymorphisms (C/C vs. C/G or G/G) and >25% decline in eGFR ($P = 0.058$). **(B)** *SLC12A3* rs16963397 polymorphisms (C/C vs. C/G or G/G) and >50% decline in eGFR ($P = 0.038$). The findings of *SLC12A3* rs16963397 C/C and *SLC12A3* rs13334864 C/C polymorphisms were the same.

findings of *SLC12A3* rs16963397 C/C and *SLC12A3* rs13334864 C/C polymorphisms were the same. Participants with the *SLC12A3* rs7187932 A/A polymorphism had similar >25 and >50% eGFR decline to those with A/G or G/G polymorphisms (log-rank test, $P = 0.340$ and $P = 0.298$, respectively).

Cox regression analysis revealed that the *SLC12A3* rs16963397 C/C and rs13334864 C/C polymorphisms were significantly associated with an increased risk of >25% decline in eGFR (HR, 3.294; 95% CI, 1.158–9.368; $P = 0.025$) and >50% decline in eGFR (HR, 18.630; 95% CI, 1.529–227.005; $P = 0.022$) than their counterparts (**Table 4**). The findings of *SLC12A3* rs16963397 C/C and *SLC12A3* rs13334864 C/C polymorphisms were the same.

DISCUSSION

This study aimed to examine the association between *SLC12A3* polymorphisms and renal function in Chinese patients with hypertension. To decrease the potential impact of diabetes mellitus, a well-known factor of renal dysfunction, this study was only conducted in non-diabetic participants. Furthermore, the association was tested in three cohorts, including a YOH cohort (cohort 1), a treatment-naïve cohort (cohort 2), and a follow-up cohort (cohort 3). *SLC12A3* rs16963397, rs13334864, and rs7187932 polymorphisms were found to be associated with eGFR in YOH and treatment-naïve hypertension. Furthermore, *SLC12A3* rs16963397 and rs13334864 polymorphisms were associated with renal function decline in participants with hypertension. As this is the first study to report the association between *SLC12A3* polymorphisms and renal function in patients with hypertension, further studies are still needed to further confirm our findings.

Although there have been some genetic studies of hypertensive nephropathy, these studies have mainly focused on the apolipoprotein L1 (*APOL1*) gene (23). Lipkowitz et al. reported that *APOL1* risk variants were significantly associated with CKD and kidney disease progression in African-American participants of the African American Study of Kidney Disease and Hypertension (AASK) trial (23). Parsa et al. further examined the effects of *APOL1* risk variants on CKD progression in the AASK trial and Chronic Renal Insufficiency (CRIC) study. The study found that *APOL1* risk variants were associated with higher rates of ESRD and progression of CKD in black patients compared to that in white patients (24). These findings suggest that genes other than *APOL1* might be related to hypertensive nephropathy in other non-African American populations.

Several studies have reported a link between *SLC12A3* gene variants and diabetic nephropathy (11–18). In a case-control study conducted in Japanese patients with diabetes, Tanaka et al. (11) reported that substitution of Arg913 with Gln in the *SLC12A3* gene might reduce the risk of developing diabetic nephropathy. In another case-control study conducted in Korean patients with diabetes, Kim et al. (12) reported that the *SLC12A3*-Arg913Gln variation was associated with ESRD caused by diabetic nephropathy. Moreover, in a case-control

TABLE 4 | *SLC12A3* gene polymorphisms and renal function decline (cohort 3).

	eGFR > 25% decline		eGFR > 50% decline	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
SLC12A3 rs16963397 (C/C vs. C/G or G/G)	3.294 (1.158–9.368)	0.025	18.630 (1.529–227.005)	0.022
Age, years	1.037 (1.005–1.070)	0.021	1.114 (0.974–1.273)	0.115
Sex (male vs. female)	0.423 (0.211–0.847)	0.015	0.078 (0.008–0.797)	0.031
Body mass index, kg/m^2	1.051 (0.967–1.142)	0.243	1.012 (0.810–1.264)	0.917
Office BP ≥ 140/90 mmHg	2.374 (1.210–4.655)	0.012	11.821 (1.558–89.687)	0.017
ACEI/ARB (yes vs. no)	0.814 (0.384–1.726)	0.591	0.244 (0.038–1.561)	0.136
Beta-Blocker (yes vs. no)	1.351 (0.665–2.746)	0.406	4.316 (0.734–25.376)	0.106
CCB (yes vs. no)	1.632 (0.709–3.756)	0.250	0.312 (0.038–2.554)	0.277
Thiazide (yes vs. no)	0.849 (0.387–1.861)	0.682	10.640 (1.535–73.734)	0.017
eGFR, mL/min/1.73 m^2	1.041 (1.025–1.057)	<0.001	1.045 (1.001–1.092)	0.045
SLC12A3 rs13334864 (C/C vs. C/T or T/T)	3.294 (1.158–9.368)	0.025	18.630 (1.529–227.005)	0.022
Age, years	1.037 (1.005–1.070)	0.021	1.114 (0.974–1.273)	0.115
Sex (male vs. female)	0.423 (0.211–0.847)	0.015	0.078 (0.008–0.797)	0.031
Body mass index, kg/m^2	1.051 (0.967–1.142)	0.243	1.012 (0.810–1.264)	0.917
Office BP ≥140/90 mmHg	2.374 (1.210–4.655)	0.012	11.821 (1.558–89.687)	0.017
ACEI/ARB (yes vs. no)	0.814 (0.384–1.726)	0.591	0.244 (0.038–1.561)	0.136
Beta-blocker (yes vs. no)	1.351 (0.665–2.746)	0.406	4.316 (0.734–25.376)	0.106
CCB (yes vs. no)	1.632 (0.709–3.756)	0.250	0.312 (0.038–2.554)	0.277
Thiazide (yes vs. no)	0.849 (0.387–1.861)	0.682	10.640 (1.535–73.734)	0.017
eGFR, mL/min/1.73 m^2	1.041 (1.025–1.057)	<0.001	1.045 (1.001–1.092)	0.045
SLC12A3 rs7187932 (G/G vs. A/A or A/G)	2.408 (0.679–8.539)	0.174	6.103 (0.499–74.585)	0.157
Age, years	1.038 (1.006–1.070)	0.019	1.084 (0.967–1.215)	0.168
Sex (male vs. female)	0.436 (0.218–0.872)	0.019	0.146 (0.021–1.023)	0.053
Body mass index, kg/m^2	1.041 (0.958–1.131)	0.348	0.978 (0.791–1.208)	0.835
Office BP ≥140/90 mmHg	2.314 (1.188–4.509)	0.014	8.275 (1.266–54.083)	0.027
ACEI/ARB (yes vs. no)	0.867 (0.408–1.843)	0.710	0.380 (0.066–2.192)	0.279
Beta-blocker (yes vs. no)	1.294 (0.638–2.622)	0.475	2.906 (0.561–15.067)	0.204
CCB (yes vs. no)	1.719 (0.748–3.948)	0.202	0.519 (0.079–3.426)	0.496
Thiazide (yes vs. no)	0.889 (0.407–1.942)	0.768	7.634 (1.301–44.806)	0.024
eGFR, mL/min/1.73 m^2	1.041 (1.025–1.057)	<0.001	1.037 (0.997–1.080)	0.071

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; BP, blood pressure.

study conducted in a Malaysian population, Abu Seman et al. (13) reported that the *SLC12A3*-Arg913Gln variation was associated with diabetic nephropathy, and that the minor 913Gln allele of *SLC12A3* confers a protective effect in diabetic nephropathy. The roles of *SLC12A3* in kidney development and progression of diabetic nephropathy were further supported by animal studies in db/db mice and zebrafish. Similarly, two case-control studies also reported that *SLC12A3*-Arg913Gln variation was significantly associated with diabetic nephropathy in the Indian population (14, 15). Furthermore, a previous case-control study (16) also reported that the *SLC12A3*-Arg913Gln variation could be used to predict the risk of ESRD in Chinese patients with type 2 diabetes. In a 10-year longitudinal study of Japanese patients with type 2 diabetes, Nishiyama et al. (17) reported that the *SLC12A3*-Arg913Gln variation was linked with albumin excretion, and that the +78A allele may be protective against the development of diabetic nephropathy. However, in a case-control study conducted in Caucasians with type 2 diabetes, Ng et al. (18) found no association between *SLC12A3* gene variants and advanced diabetic nephropathy. Although the *SLC12A3* gene was found to be associated with diabetic nephropathy in some studies, the findings were not consistent with those of other studies. It is possible that the roles of *SLC12A3* in the progression of diabetic nephropathy are influenced by genetic differences.

Our study is the first to investigate the link between *SLC12A3* genetic variations and renal function decline in patients with hypertension. In the participants with YOH (cohort 1) and treatment-naïve hypertension (cohort 2), *SLC12A3* rs16963397 C/C or C/G polymorphisms, *SLC12A3* rs13334864 C/C or C/T polymorphisms, and *SLC12A3* rs7187932 A/A or A/G polymorphisms were associated with higher eGFR than their counterparts. In the follow-up cohort (cohort 3),

participants with *SLC12A3* rs16963397 C/C and rs13334864 C/C polymorphisms were prone to develop renal function decline during an average follow-up period of 5.8 ± 1.7 years.

These findings might be explained by the hypothesis of glomerular hyperfiltration in hypertension (25–27). In cohort 1, the participants (40.9 ± 7.2 years old) were the youngest and the mean eGFR was 101.9 ± 20.8 mL/min/1.73 m^2. The eGFR in males (99.0 ± 19.3 mL/min/1.73 m^2) was at the high end of the anticipated eGFR; similarly, eGFR in females (108.4 ± 22.7 mL/min/1.73 m^2) was higher than anticipated (25). In cohort 2, the participants (53.7 ± 12.9 years old) were older than those in cohort 1, and had higher eGFR than anticipated (all: 106.7 ± 23.2 mL/min/1.73 m^2; male: 103.7 ± 17.7 mL/min/1.73 m^2; female: 109.8 ± 27.6 mL/min/1.73 m^2) (25). The participants in cohort 3 were the oldest (68.5 ± 12.9 years old); although the initial eGFR of the participants was within the range of the anticipated eGFR (all: 84.2 ± 22.2 mL/min/1.73 m^2; male: 86.0 ± 23.0 mL/min/1.73 m^2; female: 86.0 ± 24.0 mL/min/1.73 m^2) (25), participants with *SLC12A3* rs16963397 C/C and *SLC12A3* rs13334864 C/C polymorphisms had faster eGFR reduction than their counterparts. These findings suggested that *SLC12A3* polymorphisms were associated with more significant glomerular hyperfiltration in the earlier stage of hypertension (cohorts 1 and 2), and resulted in a more rapid decline in eGFR in the later stage of hypertension (cohort 3) (**Supplementary Figures 1**, **2**).

Furthermore, in cohort 1, we found that participants with *SLC12A3* rs16963397 C/C or C/G polymorphisms, *SLC12A3* rs13334864 C/C or C/T polymorphisms, and *SLC12A3* rs7187932 A/A or A/G polymorphisms had higher 24-h sodium excretion than their counterparts. Previous studies have reported that high urine sodium excretion is associated with a faster decline in renal function (28). Therefore, these findings suggest that these *SLC12A3* polymorphisms associated with higher 24-h sodium excretion may result in a faster decline in renal function. However, increased urine sodium excretion is associated with increased sodium intake (29, 30). Further studies are needed to clarify the relationship between *SLC12A3* polymorphisms, sodium intake and excretion, and renal function decline in patients with hypertension.

Study Limitations

The current study has several limitations that need to be considered. First, the sample size was relatively small. Although the *SLC12A3* rs16963397 C/C and rs13334864 C/C polymorphisms were significantly associated with an increased risk of >25% decline in eGFR (cohort 3), it might only indicate association but not cause-effect relationship and the finding might be under power. Further studies with larger sample sizes are warranted in the future, such as using the data from Taiwan biobank. Second, the renal function in the two cross-sectional cohorts (cohorts 1 and 2) was grossly intact. Further case-control studies comparing participants with intact renal function and established hypertension-related CKD are warranted. Third, it is impossible to exclude all other possible causes of renal function deterioration in addition to hypertension. Therefore, this study was conducted only in non-diabetic participants. Furthermore, the first part of the study was conducted in participants with YOH (cohort 1) to minimize possible comorbidities. Fourth, renal function could also be confounded by the use of antihypertensive drugs. Therefore, the second part of the study was conducted in participants with treatment-naïve hypertension (cohort 2) to eliminate the impact of antihypertensive drugs. Fifth, only six SNP markers in the introns of *SLC12A3* were selected in the present study, and more comprehensive genetic studies should be conducted in the future. Sixth, renal events were defined as >25 and >50% decline in eGFR in the third part of the study (cohort 3). The variation in the definition of renal function impairment across studies may limit the usefulness of comparing results between studies. Seventh, many non-genetic factors might be involved in the progression of CKD (cohort 3). However, we had tried our best to enroll only non-diabetic patients with essential hypertension and no medical history of severe diseases. We had included age, sex, BMI, office BP, and antihypertensive drugs in the multivariate analysis. Finally, our hypertensive patients were limited to Chinese patients with hypertension in Taiwan. Given that the roles of *SLC12A3* may vary among different ethnicities, further studies in other ethnic cohorts are warranted.

CONCLUSION

Genetic variations in *SLC12A3* are associated with renal function in Chinese hypertensive patients. Future investigations are mandated to elucidate the detailed pathway and identify potential therapeutic targets for prevention of renal function decline in patients with hypertension.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Academic Sinica and Taipei Veterans General Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

C-CH contributed to the conception and design of the study, data acquisition, analysis, and interpretation, and drafted and critically revised the manuscript. C-MC and C-YY contributed to the conception and design of the study, the interpretation of data, and drafted the manuscript. H-BL, P-HH, L-YL, T-CW, S-JL, W-HP, and J-WC contributed to data acquisition and drafted the manuscript. All authors gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by research grants V101B-004, V102B-024, V103C-019, V104C-025, V106C-120, V108C-151, VGHUST108-G1-3-2, VTA108-V1-7-2, V110C-058, V111C-086, V111D63-002-MY2-1, and 111EA-014 from Taipei Veterans General Hospital, Taipei, Taiwan, and by research grants MOST106-2314-B-075-040 and MOST108-2314-B-075-062-MY3 from the Ministry of Science and Technology, Taiwan.

ACKNOWLEDGMENTS

We wish to thank the National Center for Genome Medicine for technical support with the genotyping.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.863275/full#supplementary-material

REFERENCES

1. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional burden of disease. *Lancet.* (2002) 360:1347–60. doi: 10.1016/S0140-6736(02)11403-6
2. Lloyd-Jones DM, Evans JC, Larson MG, Levy D. Treatment and control of hypertension in the community: a prospective analysis. *Hypertension.* (2002) 40:640–6. doi: 10.1161/01.HYP.0000035855.44620.DA
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet.* (2005) 365:217–23. doi: 10.1016/S0140-6736(05)17741-1
4. Leiba A, Fishman B, Twig G, Gilad D, Derazne E, Shamiss A, et al. Association of adolescent hypertension with future end-stage renal disease. *JAMA Intern Med.* (2019) 179:517–23. doi: 10.1001/jamainternmed.2018.7632
5. Sui Z, Wang J, Cabrera C, Wei J, Wang M, Zuo L. Aetiology of chronic kidney disease and risk factors for disease progression in Chinese subjects: a single-centre retrospective study in Beijing. *Nephrology.* (2020) 25:714–22. doi: 10.1111/nep.13714
6. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, et al. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet.* (1996) 12:24–30. doi: 10.1038/ng0196-24
7. Mastroianni N, Bettinelli A, Bianchetti M, Colussi G, De Fusco M, Sereni F, et al. Novel molecular variants of the Na-Cl cotransporter gene are responsible for Gitelman syndrome. *Am J Hum Genet.* (1996) 59:1019–26.
8. Lemmink HH, Knoers NV, Károlyi L, van Dijk H, Niaudet P, Antignac C, et al. Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Gitelman syndrome with predominant localization to the C-terminal domain. *Kidney Int.* (1998) 54:720–30. doi: 10.1046/j.1523-1755.1998.00070.x
9. Lin SH, Cheng NL, Hsu YJ, Halperin ML. Intrafamilial phenotype variability in patients with Gitelman syndrome having the same mutations in their thiazide-sensitive sodium/chloride cotransporter. *Am J Kidney Dis.* (2004) 43:304–12. doi: 10.1053/j.ajkd.2003.10.018
10. Matayoshi T, Kamide K, Takiuchi S, Yoshii M, Miwa Y, Takami Y, et al. The thiazide-sensitive Na(+)-Cl(-) cotransporter gene, C1784T, and adrenergic receptor-beta3 gene, T727C, may be gene polymorphisms susceptible to the antihypertensive effect of thiazide diuretics. *Hypertens Res.* (2004) 27:821–33. doi: 10.1291/hypres.27.821
11. Tanaka N, Babazono T, Saito S, Sekine A, Tsunoda T, Haneda M, et al. Association of solute carrier family 12 (sodium/chloride) member 3 with diabetic nephropathy, identified by genome-wide analyses of single nucleotide polymorphisms. *Diabetes.* (2003) 52:2848–53. doi: 10.2337/diabetes.52.11.2848
12. Kim JH, Shin HD, Park BL, Moon MK, Cho YM, Hwang YH, et al. SLC12A3 (solute carrier family 12 member [sodium/chloride] 3) polymorphisms are associated with end-stage renal disease in diabetic nephropathy. *Diabetes.* (2006) 55:843–8. doi: 10.2337/diabetes.55.03.06.db05-1013
13. Abu Seman N, He B, Ojala JR, Wan Mohamud WN, Östenson CG, Brismar K, Gu HF. Genetic and biological effects of sodium-chloride cotransporter (SLC12A3) in diabetic nephropathy. *Am J Nephrol.* (2014) 40:408–16. doi: 10.1159/000368916
14. Yadav AK, Kumar V, Dutta P, Bhansali A, Jha V. Variations in CCR5, but not HFE, ELMO1, or SLC12A3, are associated with susceptibility to kidney disease in north Indian individuals with type 2 diabetes. *J Diabetes.* (2014) 6:547–55. doi: 10.1111/1753-0407.12128
15. Bodhini D, Chidambaram M, Liju S, Revathi B, Laasya D, Sathish N, et al. Association of rs11643718 SLC12A3 and rs741301 ELMO1 variants with diabetic nephropathy in south Indian population. *Ann Hum Genet.* (2016) 80:336–41. doi: 10.1111/ahg.12174
16. Zhang R, Zhuang L, Li M, Zhang J, Zhao W, Ge X, et al. Arg913Gln of SLC12A3 gene promotes development and progression of end-stage renal disease in Chinese type 2 diabetes mellitus. *Mol Cell Biochem.* (2018) 437:203–10. doi: 10.1007/s11010-017-3120-z
17. Nishiyama K, Tanaka Y, Nakajima K, Mokubo A, Atsumi Y, Matsuoka K, et al. Polymorphism of the solute carrier family 12 (sodium/chloride transporters) member 3, SLC12A3, gene at exon 23 (+78G/A: Arg913Gln) is associated with elevation of urinary albumin excretion in Japanese patients with type 2 diabetes: a 10-year longitudinal study. *Diabetologia.* (2005) 48:1335–8. doi: 10.1007/s00125-005-1785-4
18. Ng DP, Nurbaya S, Choo S, Koh D, Chia KS, Krolewski AS. Genetic variation at the SLC12A3 locus is unlikely to explain risk for advanced diabetic nephropathy in caucasians with type 2 diabetes. *Nephrol Dial Transplant.* (2008) 23:2260–4. doi: 10.1093/ndt/gfm946
19. Huang CC, Chung CM, Leu HB, Lin TH, Hung SI, Wu TC, et al. Genetic variation in C-reactive protein in ethnic Chinese population in Taiwan. *Eur J Clin Invest.* (2013) 43:449–56. doi: 10.1111/eci.12067
20. Kao TW, Huang CC, Chen JW. Optimal blood pressure for the prevention of hypertensive nephropathy in nondiabetic hypertensive patients in Taiwan. *J Clin Hypertens.* (2020) 22:1425–33. doi: 10.1111/jch.13956
21. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
22. Tsai WC, Wu HY, Peng YS, Yang JY, Chen HY, Chiu YL, et al. Association of intensive blood pressure control and kidney disease progression in nondiabetic patients with chronic kidney disease: a systematic review and meta-analysis. *JAMA Intern Med.* (2017) 177:792–9. doi: 10.1001/jamainternmed.2017.0197
23. Lipkowitz MS, Freedman BI, Langefeld CD, Comeau ME, Bowden DW, Kao WH, et al. Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. *Kidney Int.* (2013) 83:114–20. doi: 10.1038/ki.2012.263
24. Parsa A, Kao WH, Xie D, Astor BC, Li M, Hsu CY, et al. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med.* (2013) 369:2183–96. doi: 10.1056/NEJMoa1310345
25. Okada R, Yasuda Y, Tsushita K, Wakai K, Hamajima N, Matsuo S. Glomerular hyperfiltration in prediabetes and prehypertension. *Nephrol Dial Transplant.* (2012) 27:1821–25. doi: 10.1093/ndt/gfr651
26. Palatini P. Glomerular hyperfiltration: a marker of early renal damage in pre-diabetes and pre-hypertension. *Nephrol Dial Transplant.* (2012) 27:1708–14. doi: 10.1093/ndt/gfs037
27. Rossitto G, Maiolino G, Lerco S, Ceolotto G, Blackburn G, Mary S, et al. High sodium intake, glomerular hyperfiltration, and protein catabolism in patients with essential hypertension. *Cardiovasc Res.* (2021) 117:1372–81. doi: 10.1093/cvr/cvaa205

28. Deriaz D, Guessous I, Vollenweider P, Devuyst O, Burnier M, Bochud M, et al. Estimated 24-h urinary sodium and sodium-to-potassium ratio are predictors of kidney function decline in a population-based study. *J Hypertens.* (2019) 37:1853–60. doi: 10.1097/HJH.0000000000002098
29. Wielgosz A, Robinson C, Mao Y, Jiang Y, Campbell NR, Muthuri S, et al. The impact of using different methods to assess completeness of 24-hour urine collection on estimating dietary sodium. *J Clin Hypertens.* (2016) 18:581–4. doi: 10.1111/jch.12716
30. Chen SL, Dahl C, Meyer HE, Madar AA. Estimation of salt intake assessed by 24-hour urinary sodium excretion among somali adults in Oslo, Norway. *Nutrients.* (2018) 10:900. doi: 10.3390/nu10070900

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 15 September 2022
DOI 10.3389/fmed.2022.769329

OPEN ACCESS

EDITED BY
Ana Cusumano,
Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC), Argentina

REVIEWED BY
Matias Trillini,
Mario Negri Pharmacological Research Institute (IRCCS), Italy
Gaetano Alfano,
University of Modena and Reggio Emilia, Italy

*CORRESPONDENCE
Irene L. Noronha
irenenor@usp.br

SPECIALTY SECTION
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

RECEIVED 01 September 2021
ACCEPTED 24 August 2022
PUBLISHED 15 September 2022

CITATION
Noronha IL, Santa-Catharina GP, Andrade L, Coelho VA, Jacob-Filho W and Elias RM (2022) Glomerular filtration in the aging population. *Front. Med.* 9:769329. doi: 10.3389/fmed.2022.769329

Glomerular filtration in the aging population

Irene L. Noronha[1,2]*, Guilherme P. Santa-Catharina[1], Lucia Andrade[1], Venceslau A. Coelho[3], Wilson Jacob-Filho[3] and Rosilene M. Elias[1]

[1]Renal Division, Hospital das Clinicas, University of São Paulo Medical School, São Paulo, Brazil, [2]Laboratory of Cellular, Genetic and Molecular Nephrology, University of São Paulo Medical School, São Paulo, Brazil, [3]Geriatric Division, Hospital das Clinicas, University of São Paulo Medical School, São Paulo, Brazil

In the last decades, improvements in the average life expectancy in the world population have been associated with a significant increase in the proportion of elderly people, in parallel with a higher prevalence of non-communicable diseases, such as hypertension and diabetes. As the kidney is a common target organ of a variety of diseases, an adequate evaluation of renal function in the approach of this population is of special relevance. It is also known that the kidneys undergo aging-related changes expressed by a decline in the glomerular filtration rate (GFR), reflecting the loss of kidney function, either by a natural senescence process associated with healthy aging or by the length of exposure to diseases with potential kidney damage. Accurate assessment of renal function in the older population is of particular importance to evaluate the degree of kidney function loss, enabling tailored therapeutic interventions. The present review addresses a relevant topic, which is the effects of aging on renal function. In order to do that, we analyze and discuss age-related structural and functional changes. The text also examines the different options for evaluating GFR, from the use of direct methods to the implementation of several estimating equations. Finally, this manuscript supports clinicians in the interpretation of GFR changes associated with age and the management of the older patients with decreased kidney function.

KEYWORDS

glomerular filtration rate (GFR), elderly, renal function, senescence, aging, estimated GFR

Introduction

The proportion of older people in the population is increasing worldwide, reflecting profound demographics changes overall. In 2020, there were 727 million elderly individuals aged 65 years or over, representing 9.3% of the world's population—a number expected to double by 2050 (1). As a result of the increase in life expectancy, non-communicable diseases such as diabetes and hypertension have become more prevalent, with a consequent increase in the prevalence of chronic kidney disease (CKD).

Over the years, the kidneys undergo aging-related changes expressed by a decline in the glomerular filtration rate (GFR), reflecting the loss of kidney function. Anatomical and functional changes become more evident in advanced ages, accounting for the GFR decrease. The loss of kidney function with advancing age indicates a physiological decline in GFR associated with healthy aging as a natural senescence process (2), a condition difficult to distinguish from the pathological decline in GFR related to kidney diseases. Half of adults over 70 years have a GFR, either measured or estimated, of <60 mL/min/1.73 m^2 (3). However, it is still not clear whether a reduction in GFR is a consequence of the aging process or if it denotes a pathological process. The frequent comorbidities particularly present in the geriatric population that can affect kidney function, such as diabetes and cardiovascular diseases, amongst others, together with the increased prevalence of albuminuria in older subjects, and the recognition of glomerular sclerosis, tubular atrophy and vascular sclerosis in biopsy samples, represent further challenges to distinguish normal aging kidney function loss from pathological CKD. This is also important considering that CKD substantially increases the risk for cardiovascular and all-cause mortality, with low GFR correlating with worse outcome.

Since 2002, when the Kidney Disease Outcomes Quality Initiative (KDOQI) published a new chronic kidney disease (CKD) classification based on five categories of estimated glomerular filtration rate (eGFR), CKD has been defined as GFR <60 mL/min/1.73 m^2 in the absence of kidney damage, as defined by structural of functional abnormalities (4). This threshold reflects a mean loss of 50% of kidney function in the healthy adult population. The KDIGO recommendations also define a GFR below 60 mL/min/1.73 m^2 as a CKD, regardless of age (5). Although there is no precise definition of the normal range of GFR for older individuals, the use of this classification, with a fixed threshold for defining CKD based on eGFR values <60 mL/min/1.73 m^2, does not consider the physiological GFR decline with aging and may result in an overdiagnosis of CKD in the elderly (6–8). In fact, an alternative age-adapted CKD definition, stratifying the GFR threshold according to age (75, 60, and 45 mL/min/1.73 m^2 for ages younger than 40, 40–64, and 65 years or older, respectively) was proposed and analyzed in a large Canadian cohort, consisted of 127,132 individuals. Using a single, fixed eGFR threshold irrespective of age resulted in a 60% higher incidence of CKD. In this study, it is of note that 75% of individuals classified as CKD according to current fixed eGFR criteria <60 mL/min/1.73 m^2 were 65 years or older and had an eGFR of 45–59 mL/min/1.73 m^2 without significant albuminuria, raising the concern whether these individuals should be classified as CKD patients. The classification of older patients with age-related eGFR decline as having CKD is of crucial relevance, as it leads to an increase the risk of unnecessary interventions in much of this elderly population with additional implications for clinical practice and health policies (6–8).

Accurate assessment of renal function in the older population is of particular clinical significance for the diagnosis and classification of CKD, enabling tailored therapeutic interventions, and avoiding treatments of unnecessary and/or potential risk. GFR measurement can be carried out using conventional approaches through renal or plasma clearances of specific filtration markers, by measuring the marker concentration in plasma and urine or only in plasma, respectively (7). Alternatively, the use of eGFR is a more accessible approach for evaluating kidney function in a daily clinical practice. Several equations available have different performances characteristics concerning precision and accuracy and to avoid bias.

The present review is aimed at understanding the physiology of glomerular filtration in the elderly, highlighting the structural and functional changes associated with normal healthy aging leading to age related loss of kidney function. Also of interest is reviewing the different options to evaluate GFR in the elderly population to provide support to clinicians in the option and interpretation of the most appropriate method to either measure or estimate GFR.

Glomerular filtration changes in the aging kidney

The kidneys receive 20–25% of the cardiac output. Appropriate glomerular filtration occurs to excrete waste products of metabolism and to maintain internal homeostasis of water and electrolytes. The most important physiological function of renal hemodynamics is to keep the blood flow and pressure within the glomerular capillary at levels that provide adequate filtration rate with subsequent optimum tubular reabsorption and secretion functions (9). An adequate glomerular filtration rate (GFR) depends on the maintenance of the structural glomerular filtration barrier such as fenestrated endothelium, glomerular basement membrane, and podocyte and slit diaphragm besides endothelial glycocalyx and specialized glycocalyx between the foot processes and basement membrane (10).

GFR is the best parameter for measuring kidney function. GFR represents the volume of plasma filtered by functioning nephrons over a specified period. Among normal adult individuals, GFR ranges from 100 to 125 mL/min/1.73 m^2 adjusted to demographic parameters such as gender, race and age (11–17). The GFR adjustment for race is a subject of recent discussion, as reported elsewhere (18–20).

With the natural aging process, all organs, including the kidneys, are progressively affected by the decline in biological functions and progressive structural changes, regardless of the presence of disease. Indeed, in healthy individuals, age-related GFR decline has long been recognized. The first report on

the decline of GFR with age, published in 1950, describes a decrease in GFR, measured by inulin clearance, from 123 ml/min/1.73 m^2 to 65 ml/min/1.73 m^2 at the age of 90, decreasing about 8 mL/min/1.73 m^2 per decade (21, 22). The Baltimore Longitudinal Study of Aging (BLSA) reported a decrease in creatinine clearance of 0.75 mL/min/year in normal subjects aged 30–90 years followed up for a period of 14 years (23). The overall decline in GFR with age, analyzed by inulin clearance, starts at 30–40 years of age and becomes more prominent in adults older than 70 years (15, 24, 25).

Although the decrease in GFR with aging has been well-recognized, the exact estimation of the magnitude of the renal function decline with healthy aging is not yet well-established. Different methodological strategies, the use of endogenous or exogenous filtration markers to measure GFR, the equation applied to estimate GFR, the demographic representation of the healthy participants' population and the follow-up period represent issues that might account for discrepancies. Besides large geographical cohort and meta-analysis studies (11), evaluation of normal GFR has also been carried out with living kidney donor healthy individuals (12, 26–28).

Studies with living kidney donors confirmed a decrease in GFR with age, although with different decline rates. In analyzing GFR measured with ^{51}Cr-EDTA in 241 subjects 40–73 years of age, Grewal et al. found that GFR decreased by 0.91 mL/min/1.73 m^2 per year (29). Poggio et al., measuring GFR with ^{125}I-iothalamate clearances in 1,057 donors, found a decline of 1.49 $\pm$ 0.61 ml/min/1.73 m^2 per decade (12), whereas Rule et al., in a cross-sectional study including 1,203 adult living kidney donors, showed that GFR declined at a rate of 6.3 mL/min/1.73 m^2 per decade (2). Pottel et al. measured the GFR of 633 potential living kidney donors (by inulin, iohexol, or ^{51}Cr-EDTA) and confirmed a progressive decline with the age of an average of $-$0.89 ml/min/1.73 m^2 per year, corresponding to a Full Age Spectrum (FAS) equation prediction of an average decline rate of $-$0.92 ml/min/1.73 m^2 per year (30, 31).

More recently, the Renal Iohexol Clearance Survey in Tromsø 6 (RENIS-T6), a robust study including 1,594 healthy individuals from the general population (age 50–62 years) followed for more than 5 years, showed a measured GFR decline rate of 0.95 $\pm$ 2.23 ml/min/1.73 m^2 per year (32). A meta-analysis of iohexol clearance measurements in three European population-based cohorts of individuals aged 50–97 showed that the mean GFR was lower by $-$0.72 mL/min/1.73 m^2 per year for healthy men and $-$1.03 ml/min/1.73 m^2 per year for unhealthy men, and $-$0.92 mL/ min/1.73 m^2 per year for healthy women and $-$1.22 ml/min/1.73 m^2 per year for unhealthy women (33). Table 1 summarizes the demographic characteristics, age ranges of study population, the corresponding changes of GFR, and methods used to measure GFR in the different mentioned studies.

In summary, a slow decline in GFR with age is expected in healthy individuals as shown in different geographical regions (11), with a median loss of eGFR per year of ∼1 ml/min/1.73 m^2 (34).

Structural and functional changes in the aging kidney

The age-related decline in GFR is considered a physiological process after 30–40 years of age, with a more significant decline after the 70 s. With normal aging, nephron loss occurs and is detectable to some extent by the age-related decrease in GFR. Senescence causes functional and structural changes in the kidneys. Not only does the GFR decline with aging but so does the renal plasma flow, the glomerular capillary plasma flow rate, and the glomerular capillary ultrafiltration coefficient (Kf) (35–37).

Structural changes

Structural changes are associated with the senescence kidney process. With aging, the number of nephrons decreases among healthy adults (38–41). Under the age of 40, sclerotic glomeruli comprise <5% of the total, increasing thereafter, reaching as much as 30% of the glomerular population by the eighth decade, which can result in a progressive loss of 20–25% renal mass, mainly in the cortical region, in more advanced ages (42).

The loss of nephrons likely reflects a progressive degree of glomerulosclerosis, which increases with normal aging, even in healthy aging (2, 43–47). The accurate detection of the nephron number, which can only be assessed through histological analysis of kidney biopsies, is limited due to the invasive nature of this procedure. In this setting, the available information has been provided by studies carried out in the healthy kidney transplant donor population by means of biopsies obtained during the transplant surgery, enabling the analysis of kidney histomorphometry in different aged individuals (41, 48). Kidney biopsies obtained from the latter showed that nephron loss was characterized by a reduction in the total number of glomeruli at the expense of sclerotic glomeruli and non-sclerotic glomeruli. Evaluation of kidney cortical volume in this healthy population by imaging with computer tomography scans also identified a decrease in cortical volume (41). It is of note that the loss of nephrons with aging seems to be closely correlated not with the number of sclerotic glomeruli but, rather, with the decreased number of non-sclerotic glomeruli (41). A possible explanation for this apparent inconsistent discordance between nephron loss with aging and a relatively low detection of glomerulosclerosis relies on the theoretical concept that globally sclerotic glomeruli can be fully reabsorbed or may undergo changes such as atrophy and obsolescence (38). Hence, they can no longer be easily detected on tissue sections examined by light microscopy (41). This is of relevance due to the fact that the percentage of glomerulosclerosis routinely seen in kidney biopsy reports

TABLE 1 Demographic characteristics, age ranges of study population, the corresponding changes of GFR, methods used to measure GFR.

References	Sample	Demographic characteristics	Age range (years)	Changes in GFR with age	Methods used to analyze GFR
Davies et al. (21)	70 healthy individuals	100% male	24–89	GFR decline: from 122.8 to 65.3 mL/min/1.73 m^2 (decreasing about 8 mL/min/1.73 m^2 per decade)	Inulin clearance
Lindeman et al. (23)	254 healthy kidney subjects (non-proteinuric diabetes were included)	98% american predominately white	22–97	Creatinine clearence decline: 0.75 ml/min per year	Creatinine clearance
Fuiano et al. (24)	26 living kidney donors	100% males, young $n = 15$, older $n = 11$	19–32 65–76	GFR in young: 127 ± 5.8 mL/min/1.73 m^2 GFR in older: 79 ± 4 mL/min/1.73 m^2	Inulin clearance
Rule et al. (26)	365 living kidney donors	47.4% male, 80.3% white, age: 41.1 ± 11.4 yr	18–71	GFR decline: 4.9 mL/min/1.73 m^2 per decade	Iothalamate clearance
Grewal et al. (29)	428 living kidney donors (241 aged > 40 yr)	49.1% male	40–73	GFR decline: 0.91 mL/min/1.73 m^2 per decade	^{51}Cr-EDTA clearance
Poggio et al. (12)	1,057 living kidney donors	44% male, 11% African American	38.5 ± 10.4	GFR decline: 1.49 ± 0.61 ml/min/1.73 m^2 per decade	^{125}I-Iothalamate clearance
Rule et al. (2)	1,203 adult living kidney donors	42% male, 93% white	18–77	GFR decline: 6.3 mL/min/1.73 m^2 per decade	Iothalamate clearance
Kasiske et al. (27)	201 kidney donors, 203 paired controls	32% male	43.1 ± 11.9 43.4 ± 11.3	In kidney donors, GFR increased 1.47 ± 5.02 mL/min per year In controls, GFR declined 0.36 ± 7.55 mL/min per year	Iohexol
Baba et al. (28)	75,521 healthy individuals	47% male	42.8 ± 10.4	eGFR decline: 1.07 ± 0.42 ml/min/1.73 m^2per year.	3-variable Japanese equation
Pottel et al. (30, 31)	633 living kidney donors	36.8% male	20–>70	GFR decline: 0.89 ml/min/1.73 m^2 per year	Inulin, iohexol, or ^{51}Cr-EDTA
Eriksen et al. (32)	1,594 healthy individuals from the general population	49% male 42% hypertension BMI:27.2 Kg/m^2 UACR: 0.23 mg/mmol	50–62	GFR decline: 0.95 ± 2.23 ml/min/1.73 m^2 per year	Iohexol clearance
Waas et al. (34)	13,381 individuals from a German population cohort	48.7% male 23.5% obese	35–74	eGFR decline: approximately 1 ml/min/1.73 m^2 per year	eGFR calculated by CKD-EPI

from elderly patients may substantially undervalue the true age-related loss of glomeruli. The process has not been fully elucidated yet, and further studies are needed to explain the pathophysiology of age-related renal dysfunction.

Along with the structural and functional impairment changes, an age-related decline in GFR occurs with healthy aging (48–51). Progressive loss of filtering glomeruli due to glomerulosclerosis or vanished glomeruli affects the remaining preserved nephrons to undergo compensatory hypertrophy responses (52, 53), with a compensatory increase in the single-nephron GFR and glomerular capillary hydraulic pressure aiming to preserve global GFR (48). However, the enlarged glomeruli derived from compensatory hypertrophy induce an increased tension in the glomerular capillary walls, leading to hypertension, hyperfiltration, and damage to the remaining nephrons (54). Experimental studies have also shown a reduction in Kf in older rats as a result of glomerular capillary permeability and the surface area available for filtration, and confirmed an increase in glomerular capillary hydraulic pressure due to the decrease in the afferent arteriolar resistance (36).

Increased glomerular volume associated with compensatory hypertrophy can induce podocyte injury and loss. Podocytes

are crucial cells for maintaining the normal glomerular architecture and capillary permeability but, due to their terminal differentiation nature, they have a limited capacity to undergo cell division, regeneration, and repair (55). The degenerative aging phenotype along with the structural and functional changes that occur in the aging process, promote a greater podocyte detachment rate, with a decrease in the density of podocytes per glomerulus and ultimately capillary wrinkling, tuff collapse and periglomerular fibrosis (56).

The pathophysiology of renal mass decrease may be related to several mechanisms, such as a decrease in klotho expression, an increase in telomere shortening, DNA instability, increased oxygen radicals, and overexpression of proteins that induce cell cycle arrest, with induction of p16 and p21 cell cycle inhibitors (57–59). Klotho, expressed predominantly in the kidney distal convoluted tubules, has been recognized as a key modulator of aging, with antiaging effects on several pathways. The klotho deficiency that occurs with the aging process accelerates aging-related diseases, including stroke, atherosclerosis, and osteoporosis. In contrast, overexpression of the gene encoding klotho extends lifespan in mice (60). In addition, the aging process increases the oxidant activity, decreases the autophagy, and enhances the capillary rarefaction contributing to the development of renal fibrosis (59) (Figure 1).

Besides glomerulosclerosis, other structural changes become more evident in the kidneys of elderly patients, including tubular atrophy, interstitial fibrosis, arteriolar hyalinosis, and atherosclerosis that increase with aging (2). One hypothesis is that fibrointimal hyperplasia of renal arterioles occurring with advanced age triggers glomerulosclerosis and consequent interstitial fibrosis and focal atrophy (61). Indeed, said age-related histological features are challenging to distinguish from disease-related renal pathological changes. The presence of comorbidities, abnormal urinary albumin excretion, and elevated blood pressure may weigh in favor of disease-related renal pathological changes. Finally, the number of renal cysts also increases with normal aging becoming very common in the kidney parenchyma (56).

Arterial hypertension, a high-sodium diet, the Western lifestyle, and its correlated comorbidities such as obesity and diabetes have already been associated with age-related loss of kidney function, although there is evidence that this process occurs despite the presence of these factors. Studies performed in patients without arterial hypertension or in diabetic patients showed similar declines in renal function rates (62, 63), suggesting not necessarily a pathological process.

Functional changes

Several studies indicate that renal hemodynamics is impaired with aging due primarily to a vascular process. The altered renovascular tone can be due to structural lesions in the renal vasculature, more evident in older patients with cardiovascular comorbidities, whilst pronounced renal vasoconstriction is also expressed in healthy normotensive elderly subjects, possibly due to functional abnormalities (24, 64, 65). With aging, there is compromised endothelium-dependent vasodilatation in the kidney characterized by a reduced vasodilatory response to stimuli such as acetylcholine, dopamine and nitric oxide, and greater sensitivity to vasoconstrictor stimuli, such as angiotensin, norepinephrine, and endothelin (24, 64, 66, 67). The tendency toward increased renal vasoconstriction due to a defect in nitric oxide-dependent response is also responsible for sodium retention (68) and the deregulation of the pressure-natriuresis response (69). In the elderly, the renin angiotensin system is suppressed. Although renin production and release are decreased with aging, leading to lower renin and aldosterone levels, the response to these hormones is exaggerated (36).

With the aging process, other changes in kidney function can be observed (Table 2). The ability to urinary concentration or dilution is impaired with advancing age and GFR deterioration. After fluid overload, older individuals have a decreased diluting capacity due possibly to a defect to generate free water. Older individuals also have a decreased ability for urinary concentration. Compared to younger individuals, older adults have about a 50% reduced capacity to conserve water and solutes under water deprivation. Reduced thirst response to osmotic changes, and changes in plasma osmolality, make elderly subjects more susceptible to developing disturbances in water homeostasis and volume depletion (42, 70). Decreased water-conserving capacity can have important clinical implications when older individuals do not have adequate access to water (70).

Renal regulation of sodium/potassium balance is also impaired in the elderly. Individuals older than 60 years submitted to sodium restriction have a lower capacity to reduce sodium excretion. On the other hand, older individuals submitted to sodium load do not properly excrete sodium, leading to sodium retention and fluid volume expansion (42). Potassium homeostasis is also altered in aging. Animal studies demonstrated that potassium excretion was less efficient in aging rats fed with a high-potassium diet (71). In clinical settings, medications that interfere with potassium excretion should be carefully evaluated due to the propensity of older patients to develop hyperkalemia.

Aging people are also prone to developing acid-base dysregulation. The age-related decline in the GFR reduces the capacity of the kidney to buffer metabolic changes and excrete the excess H^+ load and ammonium (72).

In addition, calcium homeostasis could be affected by aging. Older individuals present impaired vitamin D production in the skin, impaired production of 1,25-(OH) 2D3, and an age-related decrease in the capacity of 1,25-dihydroxyvitamin D3 to

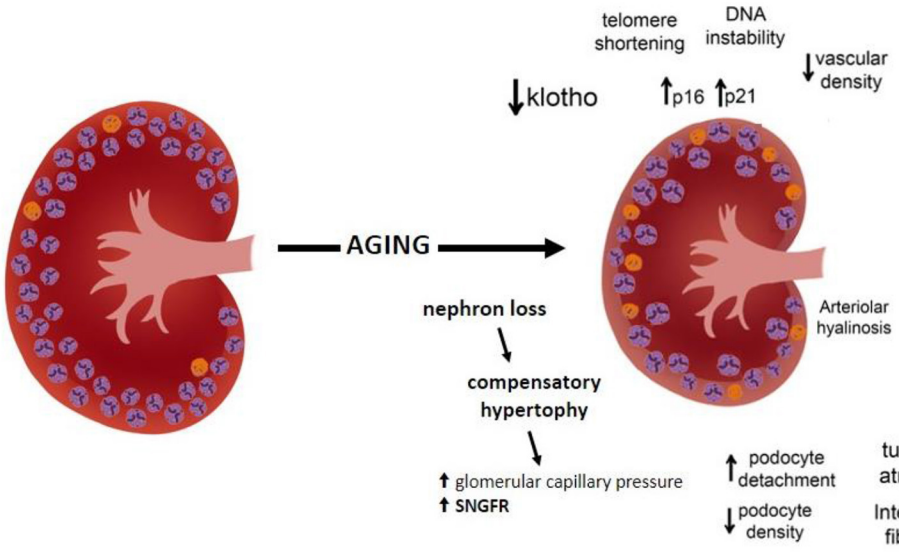


FIGURE 1
Schematic structural and functional changes in the aging kidney.

promote intestinal absorption of calcium, resulting in increased PTH secretion and higher FGF23 levels (73).

How to evaluate and interpret GFR in the elderly

Several studies have addressed the discussion of the accuracy of GFR measurements in the elderly (15, 74–78). Conventional approaches for determining measured GFR use the principle of renal clearance of various substances, including endogenous filtration markers (creatinine or cystatin C) or exogenous markers (such as inulin, iohexol, iothalamate, or other radiolabeled compounds). These substances, excreted by the kidney, should ideally be exclusively excreted by glomerular filtration with no or minimal secretion by the tubules.

GFR measurement using endogenous filtration markers

The determination of inulin clearance performed under continuous intravenous infusion and urine collection is considered the most accurate, and still the gold standard method for GFR measurement, but it is cumbersome, expensive and not feasible in daily clinical practice (79). Alternatively, renal clearance can be performed using creatinine, an endogenous product freely filtered by the glomerulus, though also secreted by tubular cells. Thus, urinary creatinine clearance, a simple and accessible method, remains a common conventional method used for renal function evaluation. However, in the elderly population, besides the potential errors associated with inadequate urine sample collection, the use of creatinine as a marker for determining GFR in this age group, characterized by progressive loss of muscle mass, represents an additional drawback. Creatinine is a nitrogenous substance derived from muscle metabolism. Reduction in muscle mass is a common aging-related condition associated with decreased appetite, decreased protein intake, reduced physical activity, and sarcopenia, causing not only reduction of muscle strength but also the loss of muscle mass, reflected as lower serum creatinine levels, which impact the creatinine-based GFR estimation. Therefore, the use of serum creatinine as a marker for estimating GFR in the elderly results in an overestimation of the renal function (80).

A disadvantage of urinary clearances for measuring GFR, particularly in the frail elderly population, is the troublesome related to urine sample collection, which can lead to errors due mainly to urinary losses and incomplete urinary bladder emptying, causing sampling errors that might jeopardize the results. In this setting, the evaluation of plasma clearances for measuring GFR provides more precise results than urinary clearances (77).

Cystatin C is a more reliable endogenous marker for the evaluation of renal function compared to creatinine. Cystatin is a substance constitutively produced by all nucleated cells. As a low molecular weight protein, cystatin is freely filtered by kidneys and catabolized in the tubules, where all metabolites are reabsorbed. Cystatin C is not dependent on muscle conditions (81) but is influenced by factors such as smoking, obesity,

TABLE 2 Kidney functional changes with aging and declining GFR.

Impairment in vasodilation response in renal hemodynamics
• Greater sensitivity to vasoconstrictor stimuli (angiotensin, norepinephrine and endothelin).
• Reduced vasodilatory response (acetylcholine, dopamine and nitric oxide).
Decreased capacity to concentrate and dilute urine
• Urinary concentration Reduced capacity to conserve water and solutes under water deprivation.
• Urinary dilution Decreased diluting capacity after fluid overload, possibly due to a defect to generate free water.
Impaired renal regulation of sodium/potassium balance
• Sodium Lower capacity to reduce sodium excretion under sodium restriction. Lower capacity to excrete sodium under sodium load, leading to sodium retention and fluid volume expansion.
• Potassium Lower capacity to decrease potassium excretion under high potassium diet
Acid-base dysregulation
• Reduced capacity to buffer metabolic changes
• Reduced capacity to excrete the excess H^+ load and ammonium
Calcium homeostasis
• Impaired vitamin D production in the skin
• Impaired production of 1,25-(OH)2D3
• Increased PTH secretion
• High FGF23 levels

and inflammation. These features make this substance a more accurate alternative for measuring glomerular filtration.

GFR measurement using exogenous filtration markers

Plasma clearances can also be performed using exogenous markers. The most widely used are the non-ionic contrast agent iohexol, particularly in Europe, and the iothalamate, more broadly used in the USA. Radiolabeled compounds such as ^{51}Cr-EDTA, ^{99m}Tc-DTPA, and ^{125}I-iothalamate have also been employed for GFR measurements with different availability in different centers. Determination of plasma clearances comprises intravenous injection of the compound and subsequent blood-sample drawings at different time-points to construct a plasma disappearance curve of the exogenous marker. Although methods based on exogenous markers likely provide higher accuracy in determining GFR, they are usually more expensive and not widely accessible in routine practice. While they have also been considered as gold standard for GFR determination in many studies, it is important to be aware that substances such as iothalamate are secreted by renal tubules and therefore, can overestimate GFR as compared to inulin clearances (79). Notwithstanding these considerations, plasma clearances are considered accurate methods of measuring GFR.

GFR estimation using equations

In clinical settings, an alternative to provide reliable and easy evaluation of kidney function in the elderly is the use of estimated GFR (eGFR) equations rather than direct measurement of GFR. eGFR based on equations is a simple and less expensive assessment, recommended by clinical guidelines from the National Kidney Foundation (NKF KDOQI) and Kidney Disease Improving Global Outcomes (KDIGO) (5, 82). Different formulas derived from large cohorts have been developed to estimate GFR in adults, based on serum creatinine or cystatin C (18, 83, 84).

The Cockcroft-Gault equation was the first equation developed for estimating creatinine clearance. In 1999, Levey and coworkers developed the first equation to estimate GFR, the Modification of Diet in Renal Disease (MDRD), normalized to 1.73 m^2 body surface area (BSA) derived from measured iothalamate-measured GFR (83). In both equations, however, the geriatric population was underrepresented. In addition, these equations are based on serum creatinine, with the concerns for older patients already addressed. Both equations have been widely worldwide used, but they usually overestimate measured GFR in older patients (77, 78, 85), although Cockcroft-Gault adjusted for BSA yields a smaller bias (77). Of note, BSA-GFR is not a good normalization index in frail patients. In such a case, a BSA-GFR is usually overestimated, impairing the fair evaluation and clinical management.

The Chronic Kidney Disease-Epidemiology Collaboration equation (CKD-EPI) (18), also developed by Levey et al., is the most common currently used equation in routine clinical practice. Although CKD-EPI was developed to improve the estimation of GFR in older patients, participants older than 65 years of age were less represented in the CKD-EPI study population (only 13% of the whole population). Compared to iohexol plasma clearance, creatinine based CKD-EPI (CKD-EPICr) in older patients also overestimates GFR (77).

Equations based on serum cystatin C levels have proved their superiority over creatinine-based formulas in the elderly (86–88). A meta-analysis of 46 cross-sectional studies confirmed that the accuracy of cystatin C is superior to creatinine-based equations (eGFRCr) (34, 78, 89–91). Alternatively, equations based on the combination of cystatin C with creatinine (CKD-EPICr+Cys) have been developed, with even better precision and accuracy compared to equations based on creatinine alone or cystatin C alone (77, 92, 93). In fact, current guidelines, besides recommending CKD-EPI equations for

estimating glomerular filtration, recommend the combination of creatinine and cystatin C (eGFRCr+Cys) as a more accurate approach (5).

To provide a more precise and accurate GFR evaluation in the elderly, the Berlin Initiative Study (BIS) analyzed a cohort of subjects aged over 70 years (77), resulting in the development of two equations designed for older individuals: the BIS1, a creatinine-based equation, and the BIS2, a creatinine and cystatin C–based equation. Both equations provided very good agreements with measured GFR, including in Chinese older subjects (94).

Other eGFR equations for the elderly population have been described such as the Caucasian, Asian, Pediatric and Adult (CAPA) (95), the Japanese equations (96, 97), in a country where the population of persons older than 65 years reached more than 25% of the total population, and the Lund-Malmö revised creatinine equation (LMRCr), developed in a Swedish cohort population population (98). LMRCr showed better performances than CKD-EPIcr. Additionally, combining LMRCr with the CAPA cystatin C equation (CAPACys), by performing the arithmetic mean of the LMRCr and CAPA equations (MEANLMR+CAPA), improved the accuracy of the GFR estimation (99).

More recently, in 2016, Pottel et al. developed, in European healthy individuals, a new eGFR equation for the elderly population, the FAS (100), based on serum creatinine. The validation was carried out comparing with measured GFR (inulin, iohexol, and iothalamate clearance). The FAS equation improved the precision and accuracy of eGFR, especially in older adults, also in chinese older individuals (101). Subsequently, besides the Full Age Spectrum creatinine equation (FASCr), its combination with cystatin C (FASCr+Cys) was proposed (31).

Table 3 summarizes the main studies evaluating eGFR against a gold-standard method to measure GFR.

For the geriatric population, which equation is more accurate to estimate GFR?

Compared to gold standard clearance methods, the currently available equations for determining eGFR have different performances related to bias, precision, and accuracy, which highlights the difficulty in defining an accurate estimation of GFR in older patients. It is important to emphasize that the Cockcroft-Gault equation is not similar to CKD-EPI or other eGFR equations.

Table 4 summarizes the results of studies comparing discrepancies of GFR estimating equations in older adults examining performances between the eGFR analyzed by metrics such as bias, precision, and accuracy. Bias represents the median difference between measured GFR and estimated GFR. It is important to identify the eGFR equation with the smallest bias. Bias with negative values indicates that the equation overestimates the GFR. Precision also represents the differences between measured GFR and estimated GFR but it is expressed as the interquartile range (IQR). Accuracy can be evaluated as P30, which represents the percentage of estimates within 30% of the measured value. The highest P30 values reflect better accuracy of eGFR compared to measured GFR.

Schaeffner and coworkers compared performances of the eGFR equations, consisting of the BS1 and BS2 equations, Cockcroft-Gault, MDRD, CKD-EPICr, and CKD-EPICys, with the gold standard iohexol plasma clearance measurement in an elderly population-based cohort [the Berlin Initiative Study (BIS)] (77). BIS equations presented the smallest median bias compared with Cockcroft–Gault, MDRD, and CKD-EPICr equations, which have a higher median bias that overestimates GFR. Accordingly, the P30 values were highest for the BIS equations, followed by the CKD-EPICys and Cockcroft–Gault equations.

The 2017 study of Bjork, a large multicenter cohort of Europeans that enrolled 3,226 individuals 74–93 years of age, validated four creatinine equations (the CKD-EPICr, BIS1Cr, LMRCr, and FASCr) compared to iohexol plasma clearance as a gold-standard method (92). LMRCr had the most stable performance compared to measured GFR. FASCr can also be a good alternative to CKD-EPICr in creatinine based estimation of GFR. The addition of cystatin C to the equations (BIS2Cr+Cys, CKD-EPICr+Cys, FASCr+Cys, and MEANLMR+CAPA) improved the performances, particularly P30 accuracy.

In 2018, Björk et al. compared the performance of GFR estimating equations using creatinine and cystatin C in a cohort of elderly patients from Iceland who participated in the AGES-Reykjavik Study (93). A subgroup of 805 Caucasian individuals, 74–93 years of age, was enrolled in the period between 2007 and 2011 to participate in a substudy that measured GFR using plasma iohexol clearance (the AGES-Kidney Study). Different GFR equations such as LMRCr MEANLMR+CAPA, FASCr, and FASCr+Cys were compared to CKD-EPICr and CKD-EPICr+Cys, All equations showed high accuracy (P30 >90%). Although there were differences in performance, none of the creatinine-based equations was clearly superior in this cohort of older individuals. However, the addition of cystatin C in creatinine-based equations improved the performance of the equations. Fan et al. (103) also analyzed the 805 participants of this AGES-Reykjavik Study, comparing the performance of the Japanese, BIS, and CAPA equations with that of the CKD-EPI equations. They concluded that none of the Japanese, BIS, and CAPA equations were superior to the CKD-EPI equations. The addition of cystatin C in the CKD-EPIcr equation improved the performance of CKD-EPICr and CKD-EPICys.

Selistre et al., in a large single-center French population (n = 2,247) aged 65–90 years with various degrees of kidney

TABLE 3 Summary of studies addressing equations to estimate glomerular filtration rate and participation of older individuals.

References	eGFR equation	Sample size	Gold-standard	Conclusion
Levey et al. (83)	MDRD	$n = 1,628$ $n = 681$ >55 yr $n = 0$ >70 yr	^{125}I-iothalamate	MDRD: more accurate than measured creatinine clearance (overestimates GFR by 19%) and Cockcroft-Gault formula (overestimates GFR by 16%).
Levey et al. (83)	CKD-EPI	$n = 8,254$ $n = 69$ >75 yr	^{125}I-iothalamate	CKD-EPI: more accurate than MDRD.
Schaeffner et al. (77)	BIS 1 (creatinine) BIS 2 (cystatin C)	$n = 610$ ≥70 years	Iohexol	BIS 2: lowest bias and smallest misclassification rate; BIS 1: smallest misclassification rate among the creatinine-based equations.
Grubb et al. (95)	CAPA	$n = 1,200$ from a Swedish cohort and $n = 413$ from a Japanese cohort	Inulin	Substandard P30 among the elderly (>80 years old)
Björk et al. (98)	LMR	$n = 850$ adults	Iohexol	Increased accuracy at measured GFR ≥90 mL/min/1.73 m^2 The LM equations cannot be recommended for use in general clinical practice
Pottel et al. (100)	FAS	$n = 6,870$ $n = 1,774$ ≥70 yr	Inulin, iohexol and iothalamate	Less biased and more accurate than CKD-EPI for older adults.

MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration; BIS, Berlin Initiative Study; CAPA, Caucasian, Asian, Pediatric and Adult; LMR, Lund-Malmö revised creatinine equation; FAS, Full Age Spectrum.

impairment, tested four different equations (CKD-EPI, BIS-1, LMR, and FAS) compared to measured GFR using inulin clearance (102). The authors tested the bias, precision, and accuracy of these equations and found that the performance of the CKD-EPI equation was not different from that of BIS-1, LMR and FAS. In patients 75 years or older with measured GFR <45 ml/min/1,73 m^2, LMR and BIS were more accurate than CKD-EPI and FAS but none of them had a superior diagnostic performance.

Recently, Yamaguchi et al. estimated GFR based both on serum creatinine and cystatin C in 19,764 individuals aged 18–103 years (mean 77.0 years) separated into two groups, <75 and ≥75 years old (the older group, $n = 12,518$, mean age 82.8 years old). The analysis of discrepancies between the CKD-EPI, Japanese, and BIS equations showed that eGFRcr was overestimated with CKD-EPI or the Japanese equation. They concluded that estimation of GFR using serum cystatin C provided more accuracy in elderly people (78).

It is worth mentioning that measured GFR is considered the gold-standard approach to access GFR. This is important because any equation used to estimate GFR may have an average error as wide as 30% or even larger, even while choosing that with higher accuracy, it has a bias that may reach 30 ml/min of difference from the measured filtration rate (104). Therefore, this should be kept in mind when interpreting studies.

Clinical management of age-related GFR changes in the elderly

Accurate determination of GFR in the older population is of crucial importance for the correct evaluation of age-related kidney dysfunction, which implies appropriate management related to the stage of kidney disease.

In clinical practice, one of the most important aspects to be considered is the adequate adjustment of the dosage of prescribed drugs to avoid overdosing. Elderly patients have often been submitted to polypharmacy and many of these medications have renal excretion, which requires dose reductions adjusted to the degree of GFR compromised, more easily calculated by the GFR equations. Whether it is a pathological or age-related reduction in GFR, the dosage of renal-eliminating drugs should be adjusted according to the GFR, to avoid potential harm.

A great number of older individuals suffer chronic and acute pain, with a particularly high prevalence of joint pains and neuralgia. Non-steroidal anti-inflammatory drugs (NSAIDs) are often prescribed for the management of pain relief in the elderly population. NSAIDs are considered nephrotoxic drugs, with the exposure associated with a high risk of GFR decline of ≥30% in individuals with eGFR <60 ml/min/1.73 m^2 (105). Hence, the prescription of NSAIDs should be done carefully in this population.

TABLE 4 Studies comparing discrepancies of GFR estimating equations and estimated creatinine clearance (Cockcroft–Gault) in older adults examining performances between the eGFR analyzed by metrics such as bias, precision, and accuracy.

	Schaeffner et al. (77)	Bjork et al. (92)	Bjork et al. (93)	Selistre et al. (102)
(A) Performances between the eGFR analyzed by median bias.				
n	610	3,226	805	2,247
Age (yr)	>70	70–89	74–93	65–90
Cohort	The BIS cohort	5 cohorts (Caucasian)	AGES-Reykjavik elderly cohort	Single center French cohort
Measured GFR	Plasma iohexol clearance	Plasma iohexol clearance	Plasma iohexol clearance	Inulin clearance
Cockcroft–Gault Cr	2.53 (−4.06 to 9.21)	n.a	n.a	n.a
MDRDCr	11.29 (3.85–17.68)	n.a	n.a	n.a
CKD-EPICr	9.69 (2.45–15.49)	3.6 (3.2–4.0)	2.7 (2.1–3.3)	−2.0 (−3.0 to −1.0)
BIS-1 (Cr)	0.80 (−5.03 to 6.11)	1.7 (1.2–2.0)	n.a.	−2.0 (−3.5 to −1.5)
LMRCr	n.a	−0.7 (−1.0 to −0.4)	−4.8 (−5.4 to −4.2)[a]	2.0 (1.5–3.5)
FASCr	n.a	0.6 (0.3–0.9)	−5.7 (−6.3 to −5.1) [a]	0.0 (−0.5 to 0.5)
CKD-EPICys	2.05 (−3.23 to 8.61)	−2.7 (−3.1 to −2.3)	n.a	n.a
FASCys	n.a	−1.1 (−1.6 to −0.8)	n.a	n.a
CAPA (Cys)	n.a	−1.4 (−1.8 to −1.0)	n.a	n.a
CKD-EPICr+Cys	n.a	−0.1 (−0.4 to 0.2)	0.6 (−0.1 to 1.2)	n.a
BIS-2 (Cr+Cys)	0.87 (−4.40 to 4.98)	−1.2 (−1.5 to −0.8)	n.a	n.a
MEANLMR+CAPA	n.a	−1.0 (−1.3 to −0.6)	−2.7 (−3.2 to −2.1) [a]	n.a
FASCr+Cys	n.a	−0.8 (−1.1 to −0.5)	−5.9 (−6.5 to −5.4) [a]	n.a
(B) Performances between the eGFR analyzed by IQR–Precision.				
CKD-EPICr	n.a	12.3 (11.9–13.0)	12.1 (11.2–13.4)	15.0 (14.5–17.0)
BIS-1 (Cr)	n.a	11.6 (11.1–12.1)	n.a	15.0 (14.0–16.5)
LMRCr	n.a	10.5 (10.1–11.0)	10.8 (10.1–11.5)[b]	14.0 (13.0–15.5)
FASCr	n.a	11.1 (10.6–11.5)	10.7 (9.9–11.9)[b]	14.0 (12.5–15.0)
CKD-EPICys	n.a	11.8 (11.3–12.5)	n.a	n.a
FASCys	n.a	12.2 (11.7–12.8)	n.a	n.a
CAPA (Cys)	n.a	11.9 (11.3–12.6)	n.a	n.a
CKD-EPICr+Cys	n.a	10.2 (9.6–10.8)	10.2 (9.0–11.1)	n.a
BIS-2 (Cr+Cys)	n.a	10.5 (10.0–11.0)	n.a	n.a
MEANLMR+CAPA	n.a	9.2 (8.8–9.6)	9.3 (8.5–10.1)[c]	n.a
FASCr+Cys	n.a	10.1 (9.7–10.7)	10.0 (9.1–10.9)[c]	n.a
(C) Performances between the eGFR analyzed by P30 accuracy.				
Cockcroft–GaultCr	87.4	n.a	n.a	n.a
MDRDCr	70.9	n.a	n.a	n.a
CKD-EPICr	77.9	76.4 (74.9–77.9)	91.7 (89.9–93.4)	77.0 (73.0–80.0)
BIS-1 (Cr)	95.1	77.5 (76.1–78.9)	n.a.	76.0 (72.5–79.5)
LMRCr	n.a	83.5 (82.2–84.8)	95.0 (93.5–96.5)[b]	80.0 (76.5–83.5)
FASCr	n.a	80.9 (79.5–82.3)	95.8 (94.4–97.2)[b]	78.5 (75.0–82.0)
CKD-EPICys	89.1	78.8 (77.3–80.4)	n.a	n.a
FASCys	n.a	80.9 (79.4–82.4)	n.a	n.a
CAPA (Cys)	n.a	80.3 (78.8–81.8)	n.a	n.a
CKD-EPICr+Cys		86.8 (85.5–88.1)	96.1 (94.8–97.4)	n.a
BIS-2 (Cr+Cys)	96.1	85.7 (84.4–87.0)	n.a	n.a
MEANLMR+CAPA	n.a	88.7 (87.5–89.9)	97.3 (96.2–98.4)[b]	n.a
FASCr+Cys	n.a	85.7 (84.4–87.1)	97.8 (96.7–98.8)[b]	n.a

[a]Significantly worse ($P < 0.05$) than corresponding CKD-EPI equation. [b]Significantly better ($P < 0.05$) than corresponding CKD-EPI equation. [c]No statistical difference ($P < 0.05$) compared with corresponding CKD-EPI equation.

The use of iodinated contrast media is another potential cause of kidney injury possibly leading to kidney adverse outcomes such as renal dysfunction characterized by abrupt GFR decline and, eventually, the need for dialysis. The use of iodine-based contrast media for diagnostic and interventional radiology for the geriatric population has exponentially increased in the last decades, in oncological settings, cardiovascular imaging examinations, and other clinical situations. Risk factors for developing contrast nephrotoxicity include pre-existing CKD, dehydration, diabetes, and the use of ACE inhibitors, diuretics, and NSAIDs, all common situations in older patients providing high susceptibility to developing worsening kidney function (106, 107). The advent of iso-osmolar and hypo-osmolar agents decreased the incidence of contrast-induced AKI. Thus, the decision on its use must be considered, evaluating the risk-benefit of the imaging examination. Prehydration and n-acetylcysteine, traditionally used as protective measures, have been questioned since the latest clinical trials and have not shown benefits in their use (108, 109).

Adjustment of drug dosing in the elderly according to GFR is widely employed in clinical settings important for avoiding harmful complications. In the aging population, type 2 diabetes is a prevalent condition, and for instance, the risk of hypoglycemia can be avoided, at least in part, by the adjustment of oral antidiabetic agents. Older patients are often affected by infections for which antimicrobials are prescribed. Therefore, the knowledge of GFR is crucial in determining the ideal dose adjustment. In situations of a moderate/severe reduction in GFR, attention must be paid to avoid toxicity, and the risk of worsening renal function is needed.

In summary, the clinical management of older individuals should be based on "do not harm." The use of contrast should be leveraged according to each indication. Besides antimicrobials, all prescriptions for older individuals should consider possible the risk of further reduction in GFR. Adjustment according to measured or estimated GFR is advised. All patients should be alert against the use of potential renal-damage drugs.

Conclusion

The population is aging. With the natural aging process, all organs, including the kidneys, undergo aging-related changes characterized by a progressively decline in biological functions and anatomical changes. The loss of kidney function due to advancing age is expressed by GFR decline. Accurate assessment of renal function in the elderly is of particular clinical importance for the appropriate evaluation and management of age-related kidney dysfunction.

The gold standard methods to measure GFR include inulin clearance and plasma clearances based on clearance of radiolabelled or non-radiolabelled exogenous markers, which are laborious, expensive and not feasible in daily clinical practice. In clinical settings, an alternative to gold standard methods still capable of easily provide reliable evaluation of kidney function in the elderly is the use of estimated GFR (eGFR) equations. Hitherto, no equation precisely mirrors the measured GFR in older patients, and therefore, the results should be meticulously interpreted. The currently available equations for determining eGFR have different performances that lead to important discrepancies even in equations specifically developed for older patients. New techniques to measure GFR should be the target of future studies. The ideal method should be cost-effective and not rely only upon serum creatinine, particularly in the elderly and frail patients with sarcopenia and loss of muscle mass. Until then, equations should be used based on either creatinine or preferentially cystatin C, when available. So far, the CKD-EPI seems to be the best choice.

Author contributions

IN planned the review article and drafted the manuscript. GS-C, LA, and RE helped to draft the manuscript. RE, LA, VAC, WJ-F, and IN gave important intellectual contributions. All authors read and approved the final version.

Acknowledgments

The authors are very grateful to Luciana Beatriz Andrade for her excellent design support in Figure 1.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. United Nations. *Department of Economic and Social Affairs. Population Division. World population ageing 2020 Highlights : Living Arrangements of Older Persons*. New York, US: United Nations (2020).

2. Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, et al. The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann Intern Med.* (2010) 159:561–7. doi: 10.7326/0003-4819-152-9-201005040-00006

3. Ebert N, Jakob O, Gaedeke J, Van Der Giet M, Kuhlmann MK, Martus P, et al. Prevalence of reduced kidney function and albuminuria in older adults: The Berlin initiative study. *Nephrol Dial Transplant.* (2017) 32:997–1005. doi: 10.1093/ndt/gfw079

4. Kidney disease outcomes quality initiative (KDOQI). Part 4. Definition and classification of stagesof chronic kidney disease. 39:S46–75. doi: 10.1053/ajkd.2002.30943

5. Andrassy KM. Comments on 'KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int.* (2013) 84:622–3. doi: 10.1038/ki.2013.243

6. O'Hare AM, Rodriguez RA, Rule AD. Overdiagnosis of chronic kidney disease in older adults—an inconvenient truth. *JAMA Intern Med.* (2021) 181:1366. doi: 10.1001/jamainternmed.2021.4823

7. Delanaye P, Schaeffner E, Ebert N, Cavalier E, Mariat C, Krzesinski JM, et al. Normal reference values for glomerular filtration rate: what do we really know? *Nephrol Dial Transplant.* (2012) 27:2664–72. doi: 10.1093/ndt/gfs265

8. Liu P, Quinn RR, Lam NN, Elliott MJ, Xu Y, James MT, et al. Accounting for age in the definition of chronic kidney disease. *JAMA Intern Med.* (2021) 181:1359–66. doi: 10.1001/jamainternmed.2021.4813

9. Neal CR, Arkill KP, Bell JS, Betteridge KB, Bates DO, Winlove CP, et al. Novel hemodynamic structures in the human glomerulus. *Am J Physiol Ren Physiol.* (2018) 315:1370–84. doi: 10.1152/ajpre

10. Schlöndorff D, Wyatt CM, Campbell KN. Revisiting the determinants of the glomerular filtration barrier: what goes round must come round. *Kidney Int.* (2017) 92:533–6. doi: 10.1016/j.kint.2017.06.003

11. Delanaye P, Jager KJ, Bökenkamp A, Christensson A, Dubourg L, Eriksen BO, et al. CKD: A call for an age-adapted definition. *J Am Soc Nephrol.* (2019) 30:1785–805. doi: 10.1681/ASN.2019030238

12. Poggio ED, Rule AD, Tanchanco R, Arrigain S, Butler RS, Srinivas T, et al. Demographic and clinical characteristics associated with glomerular filtration rates in living kidney donors. *Kidney Int.* (2009) 75:1079–87. doi: 10.1038/ki.2009.11

13. Ma Y-C, Zuo L, Chen L, Su Z-M, Meng S, Li J-J, et al. Distribution of measured GFR in apparently healthy Chinese adults. *Am J Kidney Dis.* (2010) 56:420–1. doi: 10.1053/j.ajkd.2010.05.005

14. Fliser D, Ritz E. Serum cystatin C concentration as a marker of renal dysfunction in the elderly. *Am J Kidney Dis.* (2001) 37:79–83. doi: 10.1053/ajkd

15. Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function-measured and estimated glomerular filtration rate. *N Engl J Med*. (2006) 354:2473–83. doi: 10.1056/NEJMra054415

16. Diao JA, Inker LA, Levey AS, Tighiouart H, Powe NR, Manrai AK. In search of a better equation — performance and equity in estimates of kidney function. *N Engl J Med.* (2021) 384:396–9. doi: 10.1056/NEJMp2028243

17. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine- and cystatin C–based equations to estimate GFR without race. *N Engl J Med.* (2021) 385:1737–49. doi: 10.1056/nejmoa2102953

18. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

19. Quaggin SE, Palevsky PM. Removing race from kidney disease diagnosis. *J Am Soc Nephrol.* (2021) 32:2987–9. doi: 10.1681/ASN.2021091284

20. Delanaye P, Pottel H, Glassock RJ. Americentrism in estimation of glomerular filtration rate equations. *Kidney Int.* (2022) 101:856–8. doi: 10.1016/j.kint.2022.02.022

21. Davies DF, Shock NW. Age changes in glomerular filtration rate, effective renal plasma flow, and tubular excretory capacity in adult males. *J Clin Invest.* (1950) 29:496–507. doi: 10.1172/JCI102286

22. Glassock RJ, Rule AD. The implications of anatomical and functional changes of the aging kidney: with an emphasis on the glomeruli. *Kidney Int.* (2012) 82:270–7. doi: 10.1038/ki.2012.65

23. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. *J Am Geriatr Soc.* (1985) 33:278–85. doi: 10.1111/j.1532-5415.1985.tb07117.x

24. Fuiano G, Sund S, Mazza G, Rosa M, Caglioti A, Gallo G, et al. Renal hemodynamic response to maximal vasodilating stimulus in healthy older subjects. *Kidney Int.* (2001) 59:1052–8. doi: 10.1046/j.1523-1755.2001.0590031052.x

25. Wesson G. *Physiology of the Human Kidney*. New York, NY: Grune & Stratton (1969).

26. Rule AD, Gussak HM, Pond GR, Bergstralh EJ, Stegall MD, Cosio FG, et al. Transplantation measured and estimated GFR in healthy potential kidney donors. *Am J Kidney Dis.* (2004) 43:112–9. doi: 10.1053/j.ajkd.2003.09.026

27. Kasiske BL, Anderson-Haag T, Israni AK, Kalil RS, Kimmel PL, Kraus ES, et al. A prospective controlled study of living kidney donors: three-year follow-up. *Am J Kidney Dis.* (2015) 66:114–24. doi: 10.1053/j.ajkd.2015.01.019

28. Baba M, Shimbo T, Horio M, Ando M, Yasuda Y, Komatsu Y, et al. Longitudinal study of the decline in renal function in healthy subjects. *PLoS ONE.* (2015) 10:e0129036. doi: 10.1371/journal.pone.0129036

29. Grewal GS, Blake GM, Guy's K', Thomas S. *Reference Data for 51 Cr-EDTA Measurements of the Glomerular Filtration Rate Derived From Live Kidney Donors*. London, UK: Lippincott Williams & Wilkins (2005). p. 61–65.

30. Pottel H, Delanaye P, Weekers L, Selistre L, Goffin K, Gheysens O, et al. Age-dependent reference intervals for estimated and measured glomerular filtration rate. *Clin Kidney J.* (2017) 10:545–51. doi: 10.1093/ckj/sfx026

31. Pottel H, Hoste L, Yayo E, Delanaye P. Glomerular filtration rate in healthy living potential kidney donors: a meta-analysis supporting the construction of the full age spectrum equation. *Nephron.* (2017) 135:105–19. doi: 10.1159/000450893

32. Eriksen BO, Stefansson VTN, Jenssen TG, Mathisen UD, Schei J, Solbu MD, 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) 90:404–10. doi: 10.1016/j.kint.2016.03.021

33. Eriksen BO, Palsson R, Ebert N, Melsom T, van der Giet M, Gudnason V, et al. GFR in healthy aging: an individual participant data meta-analysis of iohexol clearance in european population-based cohorts. *J Am Soc Nephrol.* (2020) 31:1602–15. doi: 10.1681/ASN.2020020151

34. Waas T, Schulz A, Lotz J, Rossmann H, Pfeiffer N, Beutel ME, et al. Distribution of estimated glomerular filtration rate and determinants of its age dependent loss in a German population-based study. *Sci Rep*. (2021) 11:10165. doi: 10.1038/s41598-021-89442-7

35. Hoang K, Tan JC, Derby G, Blouch KL, Masek M, Ma I, et al. Determinants of glomerular hypofiltration in aging humans. *Kidney Int.* (2003) 64:1417-24. doi: 10.1046/j.1523-1755.2003.00207.x

36. Weinstein JR, Anderson S. The aging kidney: physiological changes. *Adv Chronic Kidney Dis.* (2010) 17:302–7. doi: 10.1053/j.ackd.2010.05.002

37. Kielstein JT, Bode-Böger SM, Frölich JC, Ritz E, Haller H, Fliser D. Asymmetric dimethylarginine, blood pressure, and renal perfusion in elderly subjects. *Circulation.* (2003) 107:1891–5. doi: 10.1161/01.CIR.0000060496.23144.A7

38. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat Rec.* (1992) 232:194–201.

39. McNamara BJ, Diouf B, Douglas-Denton RN, Hughson MD, Hoy WE, Bertram JF, et al. comparison of nephron number, glomerular volume and kidney weight in Senegalese Africans and African Americans. *Nephrol Dial Transplant.* (2010) 25:1514–20. doi: 10.1093/ndt/gfq030

40. Hoy WE, Ingelfinger JR, Hallan S, Hughson MD, Mott SA, Bertram JF. The early development of the kidney and implications for future health. *J Dev Orig Health Dis.* (2010) 1:216–33. doi: 10.1017/S204017441000022X

41. Denic A, Lieske JC, Chakkera HA, Poggio ED, Alexander MP, Singh P, et al. The substantial loss of nephrons in healthy human kidneys with aging. *J Am Soc Nephrol.* (2017) 28:313–20. doi: 10.1681/ASN.2016020154

42. Karam Z, Tuazon J. Anatomic and physiologic changes of the aging kidney. *Clin Geriatr Med.* (2013) 29:555–64. doi: 10.1016/j.cger.2013.05.006

43. Kaplan C, Pasternack B, Shah H, Gallo G. Age-related incidence of sclerotic glomeruli in human kidneys. *Am J Pathol.* (1975) 80:227–34.

44. Anderson S, Brenner BM. Effects of aging on the renal glomerulus. *Am J Med.* (1986) 80:435–42. doi: 10.1016/0002-9343(86)90718-7

45. Kremers WK, Denic A, Lieske JC, Alexander MP, Kaushik V, Elsherbiny HE, et al. Distinguishing age-related from disease-related glomerulosclerosis on

kidney biopsy: the aging kidney anatomy study. *Nephrol Dial Transplant.* (2015) 30:2034–9. doi: 10.1093/ndt/gfv072

46. Neugarten J, Gallo G, Silbiger S, Kasiske B. Glomerulosclerosis in aging humans is not influenced by gender. *Am J Kidney Dis.* (1999) 34:884–8. doi: 10.1016/S0272-6386(99)70046-6

47. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF. A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy. *Kidney Int Suppl.* (2003) 63:S31–7. doi: 10.1046/j.1523-1755.63.s83.8.x

48. Denic A, Mathew J, Lerman LO, Lieske JC, Larson JJ, Alexander MP, et al. Single-nephron glomerular filtration rate in healthy adults. *N Engl J Med.* (2017) 376:2349–57. doi: 10.1056/nejmoa1614329

49. Roseman DA, Hwang S-J, Oyama-Manabe N, Chuang ML, O'Donnell CJ, Manning WJ, et al. Clinical associations of total kidney volume: the framingham heart study. *Nephrol Dial Transplant.* (2016) 32:1344–50. doi: 10.1093/ndt/gfw237

50. Wang X, Vrtiska TJ, Avula RT, Walters LR, Chakkera HA, Kremers WK, et al. Age, kidney function, and risk factors associate differently with cortical and medullary volumes of the kidney. *Kidney Int.* (2014) 85:677–85. doi: 10.1038/ki.2013.359

51. Denic A, Glassock RJ, Rule AD. Structural and Functional Changes With the Aging Kidney. *Adv Chronic Kidney Dis.* (2016) 23:19–28. doi: 10.1053/j.ackd.2015.08.004

52. Abdi R, Slakey D, Kittur D, Racusen LC. Heterogeneity of glomerular size in normal donor kidneys: impact of race. *Am J Kidney Dis.* (1998) 32:43–6. doi: 10.1053/ajkd.1998.v32.pm9669422

53. Fulladosa X, Moreso F, Narváez JA, Grinyó JM, Serón D. Estimation of total glomerular number in stable renal transplants. *J Am Soc Nephrol.* (2003) 14:2662–8. doi: 10.1097/01.ASN.0000088025.33462.B0

54. Daniels BS, Hostetter TH. Adverse effects of growth in the glomerular microcirculation. *Am J Physiol Ren Fluid Electrolyte Physiol.* (1990) 258:1409–16. doi: 10.1152/ajprenal.1990.258.5.f1409

55. Wiggins JE, Goyal M, Sanden SK, Wharram BL, Shedden KA, Misek DE, et al. Podocyte hypertrophy, "adaptation" and "decompensation" associated with glomerular enlargement and glomerulosclerosis in the aging rat: Prevention by calorie restriction. *J Am Soc Nephrol.* (2005) 16:2953–66. doi: 10.1681/ASN.2005050488

56. Hommos MS, Glassock RJ, Rule AD. Structural and functional changes in human kidneys with healthy aging. *J Am Soc Nephrol.* (2017) 28:2838–44. doi: 10.1681/ASN.2017040421

57. Andrade L, Rodrigues CE, Gomes SA, Noronha IL. Acute kidney injury as a condition of renal senescence. *Cell Transplant.* (2018) 27:739–53. doi: 10.1177/0963689717743512

58. Schmitt R, Cantley LG. The impact of aging on kidney repair. *Am J Physiol Ren Physiol.* (2008) 294:1265–72. doi: 10.1152/ajprenal.00543.2007.-The

59. Yang H, Fogo AB. Cell senescence in the aging kidney: figure 1. *J Am Soc Nephrol.* (2010) 21:1436–9. doi: 10.1681/ASN.2010020205

60. Kuro-o M. Klotho and aging. *Biochim Biophys Acta - Gen Sub.* (2009) 1790:1049–58. doi: 10.1016/j.bbagen.2009.02.005

61. Martin JE, Sheaff MT. Renal ageing. *J Pathol.* (2007) 211:198–205. doi: 10.1002/path.2111

62. Hollenberg NK, Rivera A, Meinking T, Martinez G, McCullough M, Passan D, et al. Age, renal perfusion and function in island-dwelling indigenous Kuna Amerinds of Panama. *Nephron.* (1999) 82:131–8. doi: 10.1159/000045389

63. Lindeman RD, Tobin JD, Shock NW. Association between blood pressure and the rate of decline in renal function with age. *Kidney Int.* (1984) 26:861–8. doi: 10.1038/ki.1984.229

64. Fliser D, Franek E, Joest M, Block S, Mutschler E, Ritz E. Renal function in the elderly: Impact of hypertension and cardiac function. *Kidney Int.* (1997) 51:1196–204.

65. Fliser D, Ritz E. Relationship between hypertension and renal function and its therapeutic implications in the elderly. *Gerontology.* (1998) 44:123–31. doi: 10.1159/000021995

66. Higashi Y, Oshima T, Ozono R, Matsuura H, Kajiyama G. Aging and severity of hypertension attenuate endothelium-dependent renal vascular relaxation in humans. *Hypertension.* (1997) 30:252–8. doi: 10.1161/01.HYP.30.2.252

67. Sabbatini M, Sansone G, Uccello F, De Nicola L, Giliberti A, Sepe V, et al. Functional versus structural changes in the pathophysiology of acute isehemic renal failure in aging rats. *Kidney Int.* (1994) 45:1355–61. doi: 10.1038/ki.1994.177

68. Baylis C. Sexual dimorphism in the aging kidney: Differences in the nitric oxide system. *Nat Rev Nephrol.* (2009) 5:384–96. doi: 10.1038/nrneph.2009.90

69. Granger A. Abnormal pressure-natriuresis in hypertension: role of nitric oxide. *Acta Physiol Scand.* (2000) 168:161–8. doi: 10.1046/j.1365-201x.2000.00655.x

70. Sands JM. Urine concentrating and diluting ability during aging. *J Gerontol Ser A Biol Sci Med Sci.* (2012) 67:1352–7. doi: 10.1093/gerona/gls128

71. Bengele HH, Mathias R, Perkins JH, McNamara ER, Alexander EA. Impaired renal and extrarenal potassium adaptation in old rats. *Kidney Int.* (1983) 23:684–90. doi: 10.1038/ki.1983.79

72. Berkemeyer S, Vormann J, Günther ALB, Rylander R, Frassetto LA, Remer T. Renal net acid excretion capacity is comparable in prepubescence, adolescence, and young adulthood but falls with aging. *J Am Geriatr Soc.* (2008) 56:1442–8. doi: 10.1111/j.1532-5415.2008.01799.x

73. Gallagher JC. Vitamin D and Aging. *Endocrinol Metab Clin North Am.* (2013) 42:319–32. doi: 10.1016/j.ecl.2013.02.004

74. Schaeffner ES, Van Der Giet M, Gaedeke J, Tölle M, Ebert N, Kuhlmann MK, et al. The Berlin initiative study: the methodology of exploring kidney function in the elderly by combining a longitudinal and cross-sectional approach. *Eur J Epidemiol.* (2010) 25:203–10. doi: 10.1007/s10654-010-9424-x

75. Garasto S, Fusco S, Corica F, Rosignuolo M, Marino A, Montesanto A, et al. Estimating glomerular filtration rate in older people. *Biomed Res Int.* (2014) 2014:916542. doi: 10.1155/2014/916542

76. Liu X, Wang Y, Wang C, Shi C, Cheng C, Chen J, et al. A new equation to estimate glomerular filtration rate in Chinese elderly population. *PLoS ONE.* (2013) 8:0079675. doi: 10.1371/journal.pone.0079675

77. Schaeffner ES, Ebert N, Delanaye P, Frei U, Gaedeke J, Jakob O, et al. Two novel equations to estimate kidney function in persons aged 70 years or older. *Ann Intern Med.* (2012) 157:471–81. doi: 10.7326/0003-4819-157-7-201210020-00003

78. Yamaguchi Y, Itabashi M, Yumura W, Takei T. Geriatric assessment of estimated glomerular filtration rate: a cross-sectional study. *Clin Exp Nephrol.* (2020) 24:216–24. doi: 10.1007/s10157-019-01797-4

79. Sterner G, Frennby B, Mansson S, Nyman U, Van Westen D, Almén T. Determining "true" glomerular filtration rate in healthy adults using infusion of inulin and comparing it with values obtained using other clearance techniques or prediction equations. *Scand J Urol Nephrol.* (2008) 42:278–85. doi: 10.1080/00365590701701806

80. Iacomelli I, Giordano A, Rivasi G, Rafanelli M, Tortù V, Cartei A, et al. Low creatinine potentially overestimates glomerular filtration rate in older fracture patients: a plea for an extensive use of cystatin C? *Eur J Intern Med.* (2021) 84:74–9. doi: 10.1016/j.ejim.2020.06.016

81. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol.* (2008) 3:348–54. doi: 10.2215/CJN.02870707

82. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National kidney foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med.* (2003) 139:137. doi: 10.7326/0003-4819-139-2-200307150-00013

83. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. Annals of internal medicine a more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med.* (1999) 130:461–70.

84. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med.* (2012) 367:20–9. doi: 10.1056/nejmoa1114248

85. Stevens LA, Coresh J, Feldman HI, Greene T, Lash JP, Nelson RG, et al. Evaluation of the modification of diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol.* (2007) 18:2749–57. doi: 10.1681/ASN.2007020199

86. Peralta CA, Katz R, Sarnak MJ, Ix J, Fried LF, De Boer I, et al. Cystatin C identifies chronic kidney disease patients at higher risk for complications. *J Am Soc Nephrol.* (2011) 22:147–55. doi: 10.1681/ASN.2010050483

87. Kazama JJ, Kutsuwada K, Ataka K, Maruyama H, Gejyo F. Serum cystatin C reliably detects renal dysfunction in patients with various renal diseases. *Nephron.* (2002) 91:13–20. doi: 10.1159/000057599

88. Rule AD, Bailey KR, Lieske JC, Peyser PA, Turner ST. Estimating the glomerular filtration rate from serum creatinine is better than from cystatin C for evaluating risk factors associated with chronic kidney disease. *Kidney Int.* (2013) 83:1169–76. doi: 10.1038/ki.2013.7

89. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: A meta-analysis. *Am J Kidney Dis.* (2002) 40:221–6. doi: 10.1053/ajkd.2002.34487

90. Willey JZ, Moon YP, Husain SA, Elkind MS V, Sacco RL, Wolf M, et al. Creatinine versus cystatin C for renal function-based mortality prediction in an elderly cohort: the northern manhattan study. *PLoS ONE.* (2020) 15:e0226509. doi: 10.1371/journal.pone.0226509

91. Shlipak MG, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, et al. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med.* (2013) 369:932–43. doi: 10.1056/NEJMoa1214234

92. Björk J, Bäck SE, Ebert N, Evans M, Grubb A, Hansson M, et al. GFR estimation based on standardized creatinine and cystatin C: a European multicenter analysis in older adults. *Clin Chem Lab Med.* (2018) 56:422–35. doi: 10.1515/cclm-2017-0563

93. Björk J, Grubb A, Gudnason V, Indridason OS, Levey AS, Palsson R, et al. Comparison of glomerular filtration rate estimating equations derived from creatinine and cystatin C: validation in the age, gene/environment susceptibility-reykjavik elderly cohort. *Nephrol Dial Transplant.* (2018) 33:1380–8. doi: 10.1093/ndt/gfx272

94. Changjie G, Xusheng Z, Feng H, Shuguang Q, Jianwen L, Junzhou F. Evaluation of glomerular filtration rate by different equations in Chinese elderly with chronic kidney disease. *Int Urol Nephrol.* (2017) 49:133–41. doi: 10.1007/s11255-016-1359-z

95. Grubb A, Horio M, Hansson LO, Björk J, Nyman U, Flodin M, et al. Generation of a new cystatin C-based estimating equation for glomerular filtration rate by use of 7 assays standardized to the international calibrator. *Clin Chem.* (2014) 60:974–86. doi: 10.1373/clinchem.2013.220707

96. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis.* (2009) 53:982–92. doi: 10.1053/j.ajkd.2008.12.034

97. Horio M, Imai E, Yasuda Y, Watanabe T, Matsuo S. Performance of GFR equations in Japanese subjects. *Clin Exp Nephrol.* (2013) 17:352–8. doi: 10.1007/s10157-012-0704-5

98. Björk J, Grubb A, Sterner G, Nyman U. Revised equations for estimating glomerular filtration rate based on the lund-malmö study cohort. *Scand J Clin Lab Invest.* (2011) 71:232–9. doi: 10.3109/00365513.2011.557086

99. Björk J, Grubb A, Larsson A, Hansson L-O, Flodin M, Sterner G, et al. Accuracy of GFR estimating equations combining standardized cystatin C and creatinine assays: a cross-sectional study in Sweden. *Clin Chem Lab Med.* (2015) 53:403–14. doi: 10.1515/cclm-2014-0578

100. Pottel H, Hoste L, Dubourg L, Ebert N, Schaeffner E, Eriksen BO, et al. An estimated glomerular filtration rate equation for the full age spectrum. *Nephrol Dial Transplant.* (2016) 31:798–806. doi: 10.1093/ndt/gfv454

101. Chai L, Wang M, Cai K, Luo Q, Yi H, Wu J. Full age spectrum equation may be an alternative method to estimate the glomerular filtration rate in Chinese patients with chronic kidney disease. *Clin Nephrol.* (2018) 89:413–21. doi: 10.5414/CN109256

102. Da Silva Selistre L, Rech DL, De Souza V, Iwaz J, Lemoine S, Dubourg L. Diagnostic performance of creatinine-based equations for estimating glomerular filtration rate in adults 65 years and older. *JAMA Intern Med.* (2019) 179:796–804. doi: 10.1001/jamainternmed.2019.0223

103. Fan L, Levey AS, Gudnason V, Eiriksdottir G, Andresdottir MB, Gudmundsdottir H, et al. Comparing GFR estimating equations using cystatin C and creatinine in elderly individuals. *J Am Soc Nephrol.* (2015) 26:1982–9. doi: 10.1681/ASN.2014060607

104. Porrini E, Ruggenenti P, Luis-Lima S, Carrara F, Jiménez A, de Vries APJ, et al. Estimated GFR: time for a critical appraisal. *Nat Rev Nephrol.* (2019) 15:177–90. doi: 10.1038/s41581-018-0080-9

105. Wan EYF, Yu EYT, Chan L, Mok AHY, Wang Y, et al. Comparative risks of nonsteroidal anti-inflammatory drugs on CKD. *Clin J Am Soc Nephrol.* (2021) 16:898–907. doi: 10.2215/CJN.18501120

106. Alsafi A, Alsafi Z, Lakhani A, Strickland NH. Changes in renal function in elderly patients following intravenous iodinated contrast administration: a retrospective study. *Radiol Res Pract.* (2014) 2014:1–4. doi: 10.1155/2014/459583

107. Marenzi G, Lauri G, Assanelli E, Campodonico J, De Metrio M, Marana I, et al. Contrast-induced nephropathy in patients undergoing primary angioplasty for acute myocardial infarction. *J Am Coll Cardiol.* (2004) 44:1780–5. doi: 10.1016/j.jacc.2004.07.043

108. Weisbord SD, Gallagher M, Jneid H, Garcia S, Cass A, Thwin S-S, et al. Outcomes after angiography with sodium bicarbonate and acetylcysteine. *N Engl J Med.* (2018) 378:603–14. doi: 10.1056/nejmoa1710933

109. Timal RJ, Kooiman J, Sijpkens YWJ, De Vries JPPM, Verberk-Jonkers IJAM, Brulez HFH, et al. Effect of no prehydration vs sodium bicarbonate prehydration prior to contrast-enhanced computed tomography in the prevention of postcontrast acute kidney injury in adults with chronic kidney disease: the kompas randomized clinical trial. *JAMA Intern Med.* (2020) 180:533–41. doi: 10.1001/jamainternmed.2019.7428

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership