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In the absence of cardiometabolic diseases, is age an independent factor in assessing renal health and filtration? A pilot study

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ABSTRACT

Introduction: Serum creatinine (sCr) is conventionally used to characterize the progressive decline in renal filtration (RF). Assessment of RF and renal health (RH) is traditionally believed to be age-dependent. However, in the absence of cardiometabolic disease (CMD), this may not be the case.
Objectives: The purpose of this study was to determine the magnitude of age as an influencing factor independent of CMD with novel markers of RH/RF in a single health assessment.
Patients and Methods: Fifty-four participants (n = 27 men; n = 27 women; age 33.4 ± 12.5 years; BMI 26.5 ± 5.5; SBP 120 ± 10.4; DBP 77.7 ± 6.7; CHOL 174 ± 30) free of CMD were recruited to assess sCr, urine creatinine (uCr), cystatin C (CyC), and urine epidermal growth factor (uEGF) to calculate estimates of RH/F via uEGF/uCr ratio (uEGFR), eGFR - modification of diet in renal disease (MDRD), CKD-EPI, and sCr/CyC eGFR.
Results: There were no significant differences between age groups (20s, 30s, 40s, 50s) in biomarkers and estimates of RH/RF, sCr ($P=0.91$), uEGF ($P=0.46$), CyC ($P=0.13$), CyC eGFR ($P=0.10$), MDRD ($P=0.12$), CKD-EPI ($P=0.80$), and sCr/CyC eGFR ($P=0.12$). Post-hoc analysis revealed uEGFR was the only significantly different variable between 40s and 50s age groups ($P=0.02$).
Conclusion: Changes in RH/RF appear to be independent of age in the absence of CMD. Indicating RH/RF could potentially be maintained in adulthood and throughout the older adult years with the continued absence of CMD.

Implication for health policy/practice/research/medical education:
The results of this study indicate that by maintaining a healthy lifestyle throughout early- to middle-aged years, free of CMDs, renal health and filtration can be maintained. Therefore, age appears to be an independent factor that has minimal influence on renal health and filtration when compared to CMDs. In addition, incorporating novel biomarkers with traditional biomarkers of renal health and filtration appears to provide a more comprehensive noninvasive assessment in evaluating kidney function.

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Original Article

Introduction

The frequency and origin of renal decline in the world population remain elusive and not fully understood (1). Traditional assessments of renal filtration (RF) include serum creatinine (sCr) clearance to calculate eGFR (estimated glomerular filtration rate), albumin concentrations, and 24-hour urine analysis. These methods are classified as indirect measures of RF, making it arduous to determine at what time point renal decline begins (2,3). More recently, the development of novel biomarkers and methods to more directly assess renal health (RH) and RF has had growing success and support (4,5). Cystatin C (CyC) has gained support during the last decade as a more accurate biomarker to assess RF than sCr due to

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it being produced from all cell types (6,7). Additionally, analyzing urine epidermal growth factor (uEGF) as a marker of RH is growing in its utilization in assisting our current understanding of renal decline (8). uEGF promotes multiple intracellular pathways, stimulating renal cell growth, survival, and replication. Due to uEGF being a protein that is produced in the loop of Henle, it is potentially a more direct marker of kidney health (9, 10). When uEGF is used in conjunction with urine creatinine (uCr), it is potentially a more direct metric to assess RH to RF when reported as a ratio (uEGF/uCr) (9).

The progressive decline in RF has long been associated with the ageing process (11). However, several risk factors independent of age may potentially have a more adverse effect on RH and RF. Cardiometabolic disease (CMD) risk factors, such as hypertension, diabetes, and hypercholesterolemia, are known to increase inflammation and oxidative stress in the systemic circulation (12,13). These processes can lead to the damaging of the vascular and renal endothelium, leading to damaged glomeruli, thus negatively influencing RH and RF (14). Due to the lack of signs and symptoms, the detection of renal decline is usually secondary to the primary reason for a hospitalization or doctor visit. Therefore, there has been difficulty in identifying a specific timeline for renal decline as well as identifying the specific mechanisms involved in the process. Thus, it is unknown whether age, independent of other risk factors, is associated with a decline in RH and RF.

Objectives

The purpose of this study was to determine the magnitude of age as an influencing factor, in the absence of cardiometabolic risk factors, in the decline of RH and RF with novel markers. We hypothesized that in the absence of CMD, there would be no differences in traditional and novel markers of RH and RF across young to middle-aged individuals.

Patients and Methods

Study design

Each participant completed a single health assessment of overall health status to quantify RH and RF. Participants arrived at the research lab after a minimum of a four hour fast but were told to consume water to maintain healthy hydration levels. Participants were instructed to abstain from exercise the day of the health assessment. Their baseline heart rate was recorded using a Polar H7 heart rate monitor (Polar, Bethpage, NY). Blood pressure was obtained manually (American Diagnostic Corporation, Hauppauge, NY) by experienced technicians. The same technicians obtained blood and urine samples under standardized conditions. The samples were used to assess cardiometabolic health. Individuals who did not meet healthy cardiometabolic health were excluded from the participation in the study.

Participants

Healthy individuals were recruited to participate in the research study. A total of fifty-four participants (n = 27 men; n = 27 women) between the ages of 20 to 60 years of age participated in the study. They were physically active (achieving the minimum exercise recommendations established by the American Heart Association), non-smokers, having never been diagnosed with cardiovascular or metabolic diseases, and were currently not taking any medication except vitamins. Participant had to regularly attend an annual physical to their primary care physician to rule out medical diagnosis. Participant demographics are provided in Table 1.

Biochemical analysis and calculations

Blood samples totaling (17 mL) each were obtained by venipuncture into the most prominent vein site in the antecubital space. All blood samples were collected into 10 mL red-top (no additive) and 7 mL purple-top (KEDTA additive) vacuum-pressured specimen tubes. Plasma tubes were allowed to clot for 30 minutes on ice. Samples were centrifuged at 3500 RPMs for 15 minutes. Serum and plasma were recovered from the red-top and purple-top tubes, respectively, and were allocated into separate 1.7 mL storage tubes and stored at -80°C until analysis.

Participants were sent to the restroom with a sterile container and asked to provide a urine sample. The specimen cup was returned to one of the study investigators. Upon collection, the sample was put on ice for 30 minutes then centrifuged for 5 minutes at 1000 RPMs. The urine samples were separated into 1.7 mL plastic storage tubes and stored at -80°C until analysis.

Table 1. Participant Demographics

	Mean	SD
Age	33.4	12.5
Height (in.)	67.6	4.3
Weight (lbs.)	171.5	35.0
Body mass index (BMI)	26.5	5.5
Systolic blood pressure (SBP) (mm Hg)	120.1	10.4
Diastolic blood pressure (DBP) (mm Hg)	77.7	6.7
Heart rate (HR) (bpm)	70.0	12.2
Glucose (mg/dL)	95.4	7.3
Total cholesterol (Chol) (mg/dL)	174.0	30.0
HDL (mg/dL)	55.0	18.0
LDL (mg/dL)	99.0	25.0
Albumin (g/dL)	4.0	0.3

Note: All values and presented as mean ± standard deviation.

Changes in RF were calculated with sCr and CyC concentrations that were measured using the Piccolo Xpress blood chemistry analyzer Comprehensive Metabolic Panel (Abaxis, Inc., Union City, CA) and a commercial ELISA kits (R&D Systems, Minneapolis, MN, and Arbor Assays, Ann Arbor, Michigan), respectively. The intra-assay precision for the ELISA kit was determined as 3.1% coefficient of variation (CV). eGFR was calculated using the four recommended equations by the National Kidney Foundation (see Table 2). uEGF concentrations were determined using a commercial ELISA kit (R&D Systems, Minneapolis, MN) with an intra-assay precision of 2.5% CV. Urine creatinine (uCr) concentrations were determined by a colorimetric detection kit (Enzo Life Sciences Inc., Farmingdale, NY). uEGF was divided by uCr and $\log_2$ transformed as a ratio to characterize changes in RH (9). All samples were thawed to room temperature prior to testing, and all samples, controls, and standards were assayed in duplicate. The optical density of wells were determined using an ELx808 absorbance microplate reader set to 450 nm (Biotek, Winooski, VT).

uEGF is expressed as a concentration and as a ratio (see equation below). uEGF ratio (uEGFR) was $\log_2$ transformed to normalize the results.

Renal Health Ratio = (uEGF/uCr) $\log_2$

Ethical issues

Prior approval for the study was obtained by our university Institutional Review Board (IRB) for research with human subjects (project # AY2018-1169). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval granted by the institution's human research committee. Eligible individuals were provided both verbal and written information regarding the research study. Participants signed and returned the informed consent document and underwent further health screening before admittance into the study.

Statistical analysis

For statistical analyses, participants were divided into four age groups (20s, 30s, 40s, and 50s). Analysis of variance (ANOVA) was performed to determine differences between group means. Pearson's coefficient of correlation (r) was used to describe the relationship between markers of RH and RF with age. Dunn's multiple comparison test post-hoc analysis was used to determine all-possible pairwise comparisons since the sample sizes were unequal. Statistical significance was set at $P \leq 0.05$. Descriptive statistics for participants are displayed as mean ± SD in Table 1. All other data are reported as mean ± SE. Data analyses were carried out using SAS software version 9.4 (SAS, Cary, North Carolina).

Results

There were no significant differences between age groups in concentrations of traditional and non-traditional markers of RH and RF. Markers of RF, sCr ($F = 0.17$, $P = 0.91$) and CyC ($F = 1.98$, $P = 0.13$) were not significantly different between any age group. Marker of RH uEGF ($F = 0.87$, $P = 0.46$) was not significantly different between age groups (Figure 1). uCr was the only biomarker that was significantly different between the 40s and 50s age groups ($F = 5.33$, $P = 0.003$). All eGFR calculations were not significantly different between age groups. Modification of Diet in Renal Diseases (MDRD) – $F = 2.08$, $P = 0.12$; Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) – $F = 0.34$, $P = 0.80$; CyC – $F = 2.81$, $P = 0.10$; and sCr/CyC – $F = 2.05$, $P = 0.12$; Figure 2). The (uEGF/uCr) $\log_2$ ratio ($F = 1.93$, $P = 0.14$) was not significantly different between age groups. Post-hoc analyses revealed the only significant difference in markers of RH and RF was between age groups 40s and 50s for uEGFR ($F = 2.87$, $P = 0.02$; Figure 3).

Statistically significant correlations were found between age and RF. Age had a significant negative correlation with eGFR as measured by the MDRD equation ($r = -0.32$, $P = 0.05$), the CyC only equation ($r = -0.39$, $P = 0.02$), and the sCr/CyC equation ($r = -0.33$, $P = 0.04$).

Discussion

Current biomarkers used to determine RH and RF in healthy and at-risk populations can be improved upon. This pilot study's findings show no significant differences

Table 2. Renal filtration eGFR Equations

	Equation
Serum creatinine (MDRD)	$eGFR = 175 * (sCr)^{-1.154} * (Age)^{-0.203} * (0.742 \text{ if female}) * (1.212 \text{ if black})$
Serum creatinine (CKD-EPI)	$eGFR = 141 * \min(sCr/\kappa, 1)\ \alpha * \max(sCr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018 \text{ (if female)} * 1.159 \text{ (if black)}$
Serum cystatin C	$eGFR = 127.7 * sCyC^{-1.17} * age^{-0.13} * (0.91 \text{ if female}) * (1.06 \text{ if black})$
Serum creatinine/cystatin C	$eGFR = 177.6 * sCr^{-0.65} * sCyC^{-0.57} * age^{-0.20} * (0.82 \text{ if female}) * (1.11 \text{ if black})$

Standard equations referenced in the literature and by National Kidney Foundation (7).
Abbreviations and definitions: α = -0.329 (females) or -0.411 (males); κ = 0.7 (females) or 0.9 (males); min = indicates the minimum of sCr/κ or 1; max = indicates the maximum of sCr/κ or 1; sCr = serum creatinine (mg/dL); sCyC = serum cystatin C.

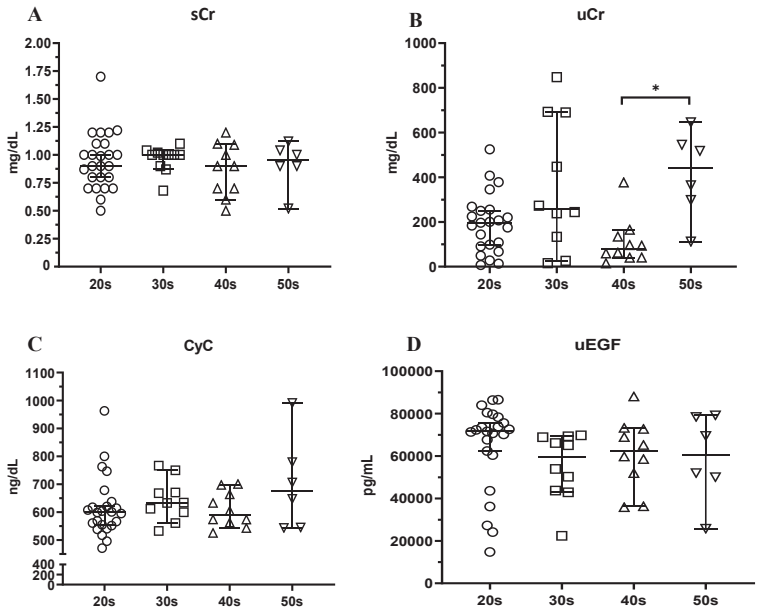

Figure 1. Traditional and Novel Biomarkers of Renal Health and Function. A) concentrations of sCr, B) concentrations of uCr, C) concentrations of serum CyC, D) concentrations of uEGF. All data are presented as mean ± SE. * Indicates significant differences between groups.

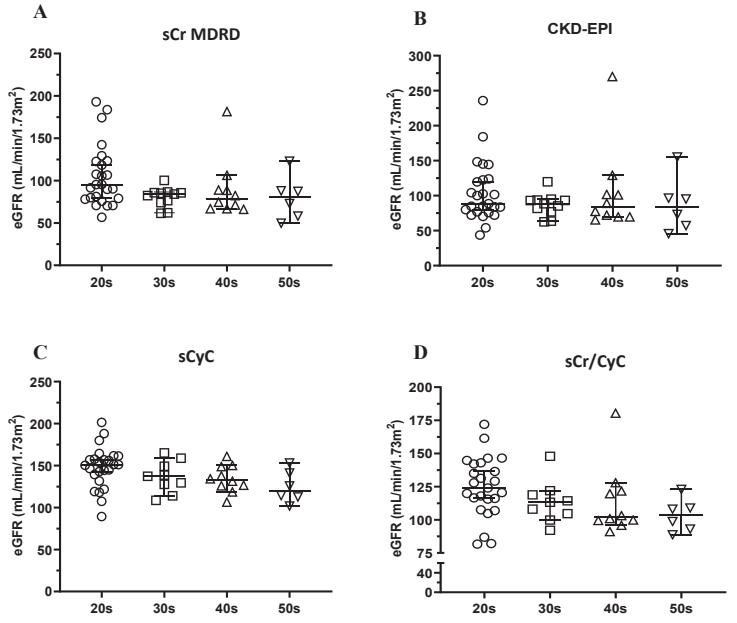

Figure 2. Standard eGFR Estimates. A) MDRD for sCr, B) CKD-EPI for sCr, C) Serum CyC, D) sCr combined with CyC. All data are presented as mean ± SE. * Indicates significant differences between groups.

in markers of RH and RF between young and middle-aged individuals in the absence of CMD. The utilization of traditional biomarkers of RH and RF in conjunction with novel markers appears to be more accurate when assessing kidney function compared to solely traditional markers. RF was negatively correlated between very young and older middle-aged individuals. The correlation has merit given the natural decline of RF. However, the rate of decline was not as sharp as previously reported in the literature (15,16). Furthermore, overall, there were no significant differences in markers of RF between the younger and older age groups.

To our knowledge, this is the first study to assess RH and RF with both traditional and novel markers in healthy young and middle-aged individuals with no CMD or risk factors. The findings of this study support our hypothesis,

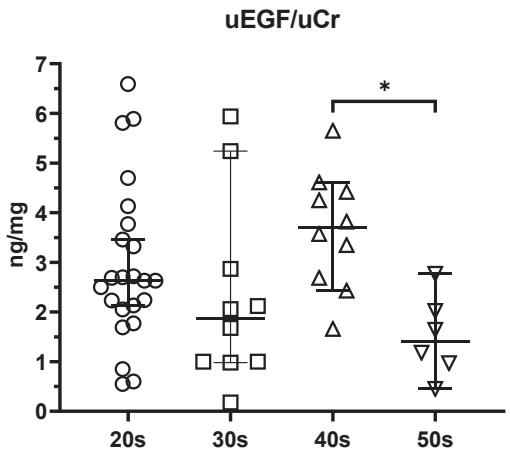

Figure 3. The ratio of uEGF to uCR to Establish Renal Health. All data are presented as mean ± SE. * Indicates significant differences between groups.

and a small segment of the literature suggesting that renal decline is more linked to other dependent factors besides being solely age-dependent (1,11,17). Declines in RH and RF have been increasing drastically worldwide over the last few decades (18). Numerous research studies have linked the reduction in RH and RF and the early development of chronic kidney disease (CKD) to CMD (19-21). On average, participants resting blood pressure, heart rate, fasting blood glucose, and cholesterol were in healthy rages, as shown in Table 1. Therefore, participants for this study were relatively free from CMD. This information further supports the idea that in the absence of CMD, there is reduced inflammation and damage to the renal vascular endothelium and glomeruli; thereby, RH and RF may be maintained for more extended periods.

The significance of the uEGF/uCr ratio is to assess RH and RF, resulting in either renal decline (lower ratio) or maintenance (higher ratio). A lower ratio indicates that the kidneys' overall health and the functional capability of the kidneys are equal or a one-to-one ratio (9). As stated in the results section, post-hoc uEGF/uCr ratio was only significantly different between age group 40s and 50s ($F = 2.87$, $P = 0.02$). Although a lower ratio was shown in the 50s age group compared to the other groups, the ratio was likely influenced by the higher concentrations of uCR. When comparing concentrations of uEGF in all groups, there were no significant differences observed ($F = 0.87$, $P = 0.46$). Thus, the results support the notion that RH and RF may be maintained for a significant period of the ageing process (Figures 2 and 3) with declines in RH beginning toward the end of middle age.

sCr remains one of the more common methods used to assess RF. However, sCr concentrations are an indirect measure of RF. Therefore, eGFR may lack accuracy in estimating RF rate (22). Using creatinine clearance alone to determine eGFR presents limitations in assessing RF. Since creatinine is produced via skeletal muscle metabolism, eGFR fails to consider other tissue and sub-tissue types; for example, epithelial, connective, and nervous tissues do not produce creatinine. Males tend to have higher production of creatinine when compared to females due to differences in muscle mass. In addition, a high percentage of muscle mass produces a greater amount of creatinine, resulting in higher serum concentrations and lower eGFR (23). The benefit of measuring CyC is that it is produced by all cell types, in all tissues, and is metabolized during glomerular filtration (24). Odutayo et al (25) recommended CyC as a marker to assess acute fluctuations in RF because CyC does not undergo renal tubular secretion. Instead, it is reabsorbed and catabolized by renal tubular cells. Similarly, CyC does not bind other blood-borne proteins and is filtered freely at the glomeruli. In general, our study results support utilizing CyC as an additional biomarker when assessing RF. When eGFR was calculated using all four recommended equations (Table 2), on average, CyC produced the highest eGFR, followed by sCr/CyC, CKD-EPI, and MDRD consistently in all groups (Figure 2). When calculating eGFR, it is important to remember that the MDRD equation significantly under predicts eGFR in healthy individuals, which we also observed in our results (16,26).

Although decreases in RH and RF are present in all ethnic populations, there is a clear gender difference in RH and RF (27,28). On average, females have a higher rate of renal decline when compared to males (29). In our study, younger males and females had similar values in their RF via eGFR. However, eGFR began to differ between males and females in the 40s group. On average, females had 7 – 25% lower eGFR when compared to males with the most substantial values coming from the traditional marker (sCr/CKD-EPI – 25%) when compared to novel marker (CyC – 7%). Differences between males and females in the marker of RH ratio, uEGF/uCr, were similar to eGFR at 23.8%. These differences are primarily thought to be influenced by hormonal changes that begin to occur in females with the onset of menopause (29). In the general population, males tend to have CKD more severely when compared to women (28). In a review, Cobo et al (27) reported that males progress to end-stage renal failure quicker than females, and are placed on dialysis more often than females. In our study, there were no gender differences observed in RH or RF in the 50s group.

More recently, the focus of clinical research has been on the prevention of more severe chronic diseases, such as CKD, congestive heart failure, cardiovascular disease, obesity, cancer, and autoimmune diseases (30). The results of our study have a high application for the prevention of CKD development. By maintaining a healthy, active

lifestyle throughout young to middle-aged years of life, an individual may significantly reduce the risk of developing CMD, protect the renal vascular endothelium from damage, and decrease the development of renal dysfunction. In addition, by incorporating novel markers of RH and RF with traditional markers, a more accurate assessment of the overall health of the kidneys can be obtained. The results potentially will aid in the prevention (lifestyle modifications) and treatment (medication therapy, diet, and exercise) process of renal decline in identified at-risk individuals.

Conclusion

In healthy individuals, changes in RH and RF appear to be independent of age in the absence of CMD. These data may indicate that RH and RF could potentially be maintained throughout adulthood, middle age, and possibly attenuated in the senior years with the continued absence of CMD. The results further support the importance of maintaining a healthy balanced lifestyle to prevent the development of CMD, which appear to have a direct influence on the decline in RH and RF. Currently, more research focus is needed on diet and exercise and their influence on RH and RF in young and middle-aged adults.

Limitations of the study

Limitations of this pilot study include the number of participants in two of the groups and the overall sample size. Another limitation is not assessing individuals over the age of sixty-one. The focus was on ages where CMD begin to manifest and have early influences on RH and RF. Additionally, we did not monitor the participant's diet or exercise in the days leading up to the health assessment. However, we did account for nutritional intake and exercise on the day of the assessment.

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Authors' contribution

JF and PK were the principal investigators of the study. JF, PK, AI, DB, and KA were included in preparing the concept and design. JF, PK, AI, DB, KA, CL, RT, and AD revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest

All authors report they have no conflict of interest to report.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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