Hypertension Research (2016) 39, 138–143

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ORIGINAL ARTICLE

Renal glomerular dysfunction in relation to retinal arteriolar narrowing and high pulse pressure in seniors

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Retinal arteriolar narrowing and high pulse pressure (PP) are associated with macrovascular complications and microvascular renal disease. Few studies addressed whether in seniors (⩾60 years) estimated glomerular filtration rate (eGFR) is independently related to central retinal arteriolar equivalent (CRAE) and PP. In 292 randomly recruited seniors (49.3% women; mean, 68.2 years), we measured PP by standard sphygmomanometry, CRAE (IVAN software), eGFR (Chronic Kidney Disease Epidemiology Collaboration equation) and stage of chronic kidney disease (CKD (Kidney Disease Outcomes Quality Initiative guideline)). Statistical methods included linear and logistic regression. PP, CRAE and eGFR averaged 59.2 mm Hg, 146.3 μm and 79.9 ml min^{-1} per 1.73 m^2. Decline in eGFR (–2.27 ml min^{-1} per 1.73 m^2 per 15 μm; $P=0.011$) occurred in parallel with CRAE narrowing. CRAE (effect size per 1-s.d. increment, –1.85 μm; $P=0.032$) and eGFR (–2.68 ml min^{-1} per 1.73 m^2; $P=0.003$) both declined with higher PP. With PP increasing from 63 to 73 mm Hg (threshold for macrovascular complications), CRAE dropped by –4.70 μm ($P\leqslant0.037$). A 70-mm Hg PP threshold corresponded with a 150-μm CRAE cutoff. The risk of CKD (stage ⩾2 *vs.* 1; $n=203$ *vs.* 89) rose with CRAE <150 μm (odds ratio, 2.81; $P<0.0001$), but not with PP ⩾70 mm Hg (1.47; $P=0.20$). Additionally, CRAE added to PP increased the area under the curve from 0.58 to 0.64 ($P=0.047$) for identifying stage ⩾2 CKD. In seniors, CRAE and eGFR decline in parallel with higher PP. CRAE <150 μm identifies early decline in eGFR.
Hypertension Research (2016) **39,** 138–143; doi:10.1038/hr.2015.125; published online 12 November 2015

Keywords: Central retinal arteriolar equivalent; elderly; glomerular filtration rate; microcirculation; population science; pulse pressure

INTRODUCTION

Both kidney and brain[1] are perfused at high-volume flow with pulsatility maintained up to the effluent venous blood flow. The microvasculature in the retina, being an extension of the brain, also shows pulsatility.[2] Narrowing of the retinal arterioles not only predicts macrovascular complications, such as coronary heart disease[3] and stroke,[4] but also progression of chronic kidney disease (CKD).[5] Recent studies in patients[6–8] and populations[9,10] demonstrated that glomerular filtration, a microvascular phenotype, is inversely correlated with indexes reflecting stiffness of the large arteries, including pulse pressure.[6,7,9,10]

We previously reported that over the whole adult age range retinal arteriolar diameter was not associated with pulse pressure, once mean arterial pressure was accounted for.[11] However, the prognostic significance of pulse pressure differs according to age.[12,13] To our knowledge, only two previous reports of the Cardiovascular Health Study presented analyses of the retinal microvasculature in relation to renal function and cardiovascular outcomes in seniors.[14,15] We therefore reassessed specifically in older participants enrolled in the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO),[11] (i) whether retinal and renal microvascular traits are associated with pulse pressure, (ii) and whether the retinal microvasculature can be an indicator of early renal dysfunction over and beyond pulse pressure.

METHODS

Study population

FLEMENGHO was conducted according to the principles outlined in the Declaration of Helsinki for Investigation of Human Participants.[16] The Ethics Committee of the University of Leuven approved the FLEMENGHO study.[17,18] Recruitment started in 1985 and continued until 2004. The initial participation rate was 78.0%. The participants were repeatedly followed up.[17,18] From January 2008 until January 2012, we invited 1574 former participants by mail for a follow-up examination. However, 168 were unavailable as they had died earlier ($n=68$), had been institutionalized or were too ill ($n=42$) or as they had moved out of the area ($n=58$). Of the remaining 1406 former participants, 1143 renewed informed consent. The participation rate was therefore 81.3%.

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Received 20 July 2015; revised 23 September 2015; accepted 15 October 2015; published online 12 November 2015

For the present analysis, we excluded 851 participants, because they were younger than 60 years ($n=774$), because the retinal images were of low quality ($n=73$) or because retinal arteriolar diameter was >3 s.d. higher than the mean ($n=4$). Thus, the number of participants statistically analyzed totaled 292.

Clinical and biochemical measurements

After participants had rested in the sitting position for at least 5–10 min, trained observers performed five consecutive blood pressure readings to the nearest 2 mm Hg by auscultation of the Korotkoff sounds.[19] The five blood pressure readings were averaged for analysis. Pulse pressure was systolic minus diastolic blood pressure. Hypertension was a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic or use of antihypertensive drugs. The observers administered a standardized questionnaire to collect information on medical history, smoking and drinking habits, and intake of medications. Body mass index was weight in kilograms divided by the square of height in meters.

With participants fasting for at least 6 h, venous blood samples were drawn for measurement of plasma glucose and serum cholesterol and creatinine. Diabetes was the use of antidiabetic drugs or a fasting glucose concentration of at least 7.0 mmol l^{-1}.[20] We measured the concentration of creatinine in serum, using Jaffe's method with modifications described elsewhere,[21] on automated analyzers in a single-certified laboratory. We derived the estimated glomerular filtration rate (eGFR) from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[22] CKD stages, defined according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guideline,[23] were eGFR $\geqslant 90$, 60–89, 45–59, 30–44, 15–29 and <15 ml min^{-1} per 1.73 m^2 for stage 1, 2, 3A, 3B, 4 and 5, respectively.

Retinal microvascular diameters

As described in detail before, we applied a non-mydriatic approach in a dimly lit room to obtain retinal photographs, one per eye in each participant, with the Canon Cr-DGi retinal visualization system combined with the Canon D-50 digital camera (Canon Inc., Medical Equipment Group, Utsunomiya City, Tochigi, Japan). Two observers determined the central retinal arteriolar (CRAE) and venular (CRVE) equivalent, which represent the average retinal arteriolar and venular diameters. For each participant, values of the two eyes were averaged. They used the validated computer-assisted program IVAN (Vasculomatic ala Nicola, version 1.1, Department of Ophthalmology and Visual Science, University of Wisconsin-Madison, Madison, WI, USA)[24] based on formulae published by Parr and Spears[25,26] and Hubbard *et al.*[27] The intraobserver variability of CRAE and CRVE was 13.2% and 8.4% for observer 1 and 9.6% and 5.1% for observer 2. The interobserver variability was 10.6% and 8.0%, respectively.

Statistical analysis

For database management and statistical analysis, we used the SAS system, version 9.3 (SAS Institute Inc., Cary, NC, USA). For comparison of means and proportions, we used Student's *t*-test or analysis of variance and Fisher's exact test, respectively. Statistical significance was a two-sided *P*-value of <0.05. In multivariable-adjusted analyses, we standardized CRAE to the average of the distributions (ratio or mean) in the whole population of covariables identified in our previous studies,[11,28] that is, sex, age, diastolic blood pressure and smoking. Previous IDACO analyses[13] suggested that risk carrying cutoff limits of pulse pressure in older people were within a 10 mm Hg range encompassing 63 and 73 mm Hg. We checked the impact on CRAE of 1-mm Hg increments in the pulse pressure within the 63–73 mm Hg range by assessing at each step the decrease in CRAE and explained variance of CRAE. To obtain corresponding thresholds for CRAE, we applied the regression method.[29] Next, turning to CKD stages as outcome and using CRAE and pulse pressure as continuous variables, we constructed receiver-operating characteristic plots. Finally, we assessed the odds of having renal dysfunction in relation to the CRAE and pulse pressure thresholds derived in the first part of our analysis.

RESULTS

Characteristics of participants

Table 1 lists the characteristics of participants by tertiles of the pulse pressure distribution. Age, systolic pressure, mean arterial pressure, the prevalence of hypertension and treated hypertension increased with higher category of pulse pressure ($P\leqslant 0.0023$), whereas the opposite was the case for diastolic pressure, pulse rate and the prevalence of smoking ($P\leqslant 0.047$). There were no differences across the pulse

Table 1 Characteristics of participants by thirds of the pulse pressure distribution

Characteristics	*< 50.4 mm Hg* (n = *97*)	*50.4–67.2 mm Hg* (n = *100*)	*> 67.2 mm Hg* (n = *95*)
Number (%) with characteristics			
Women (%)	48 (49.5)	45 (45.0)	51 (53.7)
Smokers (%)	18 (18.6)	8 (8.0)	8 (8.4)
Drinking alcohol (%)	67 (69.1)	66 (66.0)	62 (65.3)
Hypertension (%)	56 (57.7)	72 (72.0)	91 (95.8)
Antihypertensive treatment (%)	40 (41.2)	53 (53.0)	63 (66.3)
Diabetes mellitus (%)	1 (2.1)	1 (1.0)	3 (3.2)
Cardiovascular disease (%)	11 (11.3)	11 (11.0)	9 (9.5)
Mean (± s.d.) of characteristics			
Age (years)	65.7 ± 5.1	67.0 ± 6.0	72.0 ± 6.2
Body mass index (kg m^{-2})	28.1 ± 4.3	28.1 ± 4.4	28.0 ± 4.4
Systolic blood pressure (mm Hg)	126.9 ± 9.8	139.6 ± 8.9	157.8 ± 11.7
Diastolic blood pressure (mm Hg)	84.5 ± 9.1	81.7 ± 7.8	80.0 ± 9.8
Mean arterial pressure (mm Hg)	98.6 ± 8.9	101.0 ± 7.9	106.0 ± 9.5
Pulse pressure (mm Hg)	42.3 ± 5.8	57.9 ± 4.5	77.8 ± 9.2
Pulse rate (beats per min)	64.2 ± 8.6	62.3 ± 9.7	61.4 ± 8.6
Total cholesterol (mmol l^{-1})	4.86 ± 0.89	4.89 ± 0.99	4.93 ± 0.91
HDL-to-total cholesterol ratio	0.29 ± 0.07	0.30 ± 0.08	0.31 ± 0.09
Plasma glucose (mmol l^{-1})	5.10 ± 0.91	5.13 ± 1.12	5.25 ± 0.97
eGFR (ml min^{-1} per 1.73 m^2)	82.6 ± 14.6	80.1 ± 14.8	77.0 ± 16.1

Abbreviations: eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein.
eGFR was derived from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation. Blood pressure was the average of five consecutive readings. Hypertension was an office blood pressure of $\geqslant 140$ mm Hg systolic or $\geqslant 90$ mm Hg diastolic, or use of antihypertensive drugs. Diabetes was the use of antidiabetic drugs or a fasting glucose concentration of $\geqslant 7.0$ mmol l^{-1}.

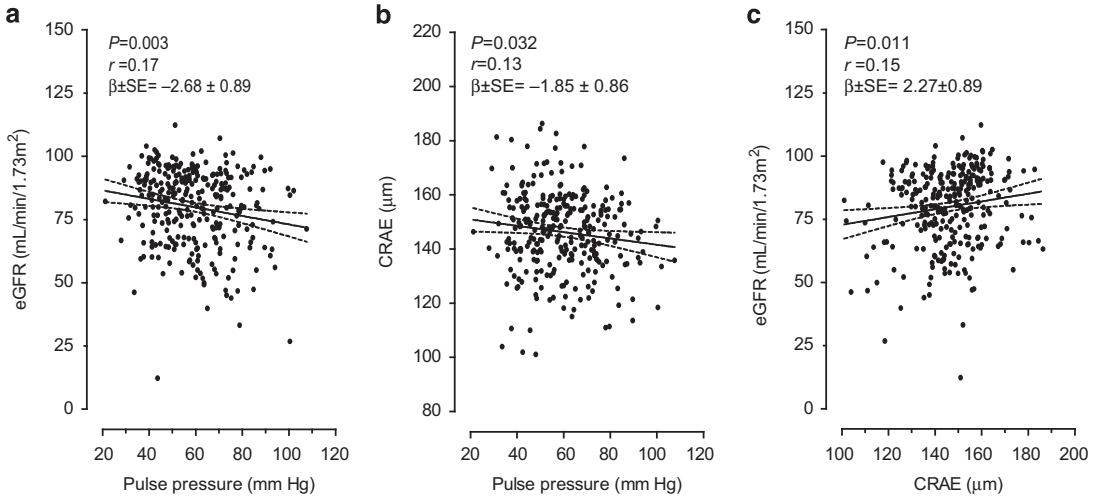

Figure 1 Linear associations of estimated glomerular filtration rate (eGFR) with pulse pressure (**a**), central retinal arteriolar equivalent (CRAE, **b**) with pulse pressure and eGFR with CRAE (**c**). The solid and dotted lines represent the regression line and 95% confidence limits, respectively.

pressure categories in body mass index, total cholesterol, high-density lipoprotein-to-total cholesterol ratio, plasma glucose, and the proportion of women, alcohol drinkers and patients with history of cardiovascular disease and diabetes ($P \geqslant 0.079$).

Continuous analyses of eGFR, CRAE and pulse pressure

In the whole study population, eGFR averaged (± s.d.) 79.9 ± 15.3 ml min^{-1} per 1.73 m^2, CRAE crude 146.3 ± 14.8 μm, CRAE standardized 146.3 ± 14.1 μm and pulse pressure 59.2 ± 15.9 mm Hg. eGFR and CRAE were inversely correlated with pulse pressure (Figure 1). Per 1-s.d. increment in pulse pressure, the decrease in eGFR amounted to 2.68 ml min^{-1} per 1.73 m^2 (95% confidence interval (CI), 0.94–4.42; $P = 0.003$; Figure 1a). The effect size for a 1-s.d. increase in pulse pressure was –1.85 μm (95% CI, –3.54 to –0.17 μm; $P = 0.032$) for crude CRAE (Figure 1b) and –1.87 μm (CI, –3.74 to –0.03 μm; $P = 0.046$) for standardized CRAE. In addition, there was a positive association between eGFR and CRAE. Per 1-s.d. drop in CRAE, eGFR declined by 2.27 ml min^{-1} per 1.73 m^2 (CI, 0.53–4.01; $P = 0.011$; Figure 1c). Sensitivity analyses accounting for plasma glucose, diabetes or pulse rate were confirmatory.

Equivalent thresholds for CRAE and pulse pressure

We determined multivariable-adjusted differences in CRAE between participants with a pulse pressure level over *vs.* below thresholds successively increasing in steps of 1 mm Hg (Figure 2a). For pulse pressure cutoffs increasing from 63 to 73 mm Hg (threshold for macrovascular complications;[13] Figure 2a), the differences in CRAE ranged from –2.48 μm (CI, –5.82 to 0.85 μm) to –4.70 μm (CI, –8.78 to –0.62 μm). These differences reached and maintained significance ($P \leqslant 0.037$) for pulse pressure thresholds encompassing 69 and 73 mm Hg. Next, we computed the partial coefficients of determination (r^2) relating CRAE with pulse pressure in participants exceeding the stepwise increasing pulse pressure thresholds (Figure 2b). Estimates of r^2 mirrored the findings in Figure 2a, in that at a pulse pressure of 69 mm Hg (up to 73 mm Hg), the variance of CRAE explained by pulse pressure in the multivariable-adjusted models increased from ~1% below 69 mm Hg to 2% at and above this threshold. We therefore set the pulse pressure threshold associated with retinal arteriolar narrowing at 70 mm Hg. Finally, we used the regression method to derive thresholds for the crude and standardized CRAE corresponding with a pulse pressure of 70 mm Hg (Figure 3). The so-obtained CRAE cutoff limits were 145.1 μm (CI, 143.0–147.1 μm) and 145.3 μm (CI, 143.4–147.3 μm). For further analyses based on thresholds, we rounded the upper limit of CRAE to 150 μm.

Risk-based analyses

Among all participants, 89, 170 and 33 had CKD stage 1, 2 and 3 or more, respectively. CRAE decreased across increasing CKD categories ($P = 0.0005$), averaging 150.5 ± 13.5 μm crude and 148.9 ± 13.2 μm standardized at CKD stage 1, 145.2 ± 14.9 μm and 145.7 ± 14.2 μm at stage 2 and 141.1 ± 14.9 μm and 142.6 ± 15.3 μm at stage 3 and beyond. In receiver-operating characteristic analyses, in which pulse pressure and CRAE were entered as continuous variables (Figure 4), CRAE added to pulse pressure in discriminating CKD stage 1 from stage 2 or higher. The area under the curve increased from 0.58 to 0.64 ($P = 0.047$; Figure 4). Along similar lines, the 150-μm CRAE threshold, but not the 70-mm Hg pulse pressure threshold, discriminated CKD stage 1 from stage 2 and beyond. The odds ratios were 2.81 (CI, 1.68–4.69; $P < 0.0001$) for CRAE (< 150 μm) and 1.47 (CI, 0.82–2.66; $P = 0.20$) for pulse pressure (⩾70 mm Hg) and when considered together in the same model, 2.74 (CI, 1.63–4.59; $P = 0.0001$) and 1.29 (CI, 0.70–2.37; $P = 0.42$), respectively. The CRAE and pulse pressure thresholds did not differentiate stage 2 or below from stage 3 or beyond ($P \geqslant 0.28$).

DISCUSSION

In people aged 60 years or older, pulse pressure predicts macrovascular complications.[13,30] This study attempted to extend these findings to microvascular traits including retinal arteriolar narrowing and impaired renal function. The key findings of our current study were (i) that in contrast to our previous findings encompassing the whole age range,[11] retinal arterioles narrow with higher pulse pressure in seniors; (ii) that in line with the literature[6–10] eGFR declines with pulse pressure; (iii) that renal microcirculatory function as captured by eGFR or CKD stage declines in parallel with retinal arteriolar narrowing; and (iv) that in seniors a CRAE of <150-μm identifies early CKD over and beyond a 70-mm Hg pulse pressure threshold.

Several cross-sectional[31–34] and prospective[3–5,14,15,35–39] studies in the general population,[3–5,14,15,31,33–39] or in patients with acute

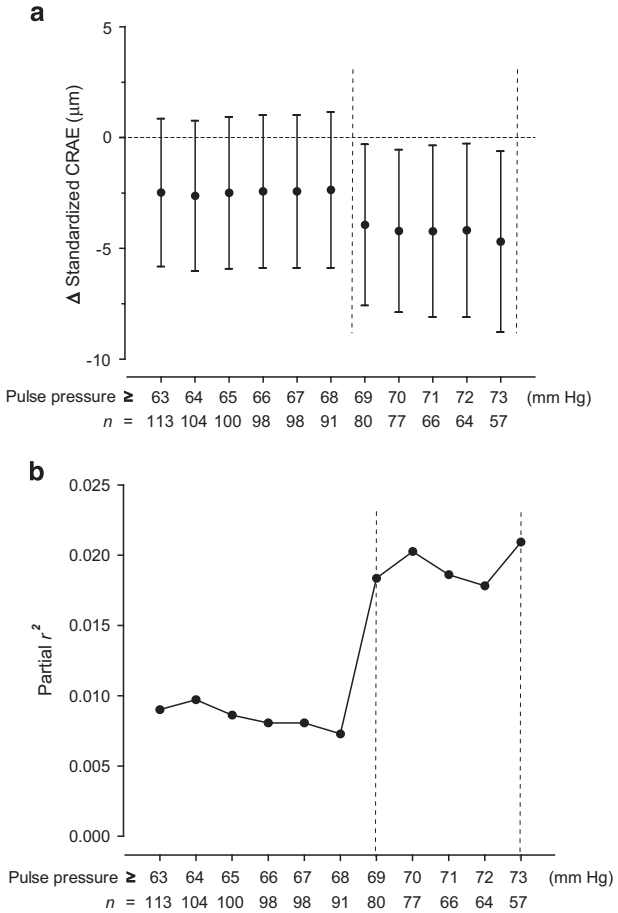

Figure 2 Differences in standardized central retinal arteriolar equivalent (CRAE) between participants with pulse pressure above *vs.* below thresholds (**a**). CRAE was standardized to the average of the distributions (ratio or mean) in the whole study population of sex, age, diastolic blood pressure and smoking. *n* is the number of participants with pulse pressure above the successive thresholds. Vertical bars denote 95% confidence intervals. With pulse pressure increasing in steps of 1 mm Hg, the CRAE differences reached and maintained significance ($P \leqslant 0.036$) for pulse pressure thresholds ranging from 69 to 73 mm Hg, but not at levels of 68 mm Hg or lower ($P \geqslant 0.077$). (**b**) The corresponding partial r^2 associated with the pulse pressure thresholds.

stroke[32] addressed the association of microvascular complications in the kidney,[5,31,33,34,39] brain[4,32,36,38] or heart[3,15,35,37] with retinal arteriolar narrowing. In the context of this manuscript, in which we studied the association between glomerular function and CRAE and in which we attempted to define a CRAE threshold reflecting early microvascular disorder, especially articles in which the CRAE distribution was subdivided into tertiles,[5] quartiles[15,31,33,34,38,39] or quintiles,[3,4,32,35–37] are relevant. In these articles, bottom and top quantiles were compared. All currently reviewed studies included approximately equal proportions of women and men. In only two articles, the participants were seniors. Among 1394 elderly aged 65 years or more (mean age, 78 years), enrolled in the Cardiovascular Health Study, participants with retinopathy showed a significant increase in serum creatinine level and decline in eGFR compared with those without retinopathy during the 4-year study period.[14] Among 1992 people from the same cohort,[15] smaller CRAE predicted coronary heart disease (rate ratio of bottom *vs.* top quartile, 2.0; 95% CI, 1.1–3.7), but not stroke (1.1; 95% CI, 0.5–2.2).

Among the remainder of the studies,[3–5,31–39] mean age ranged from 48.8[33] to 67.8 years.[38] The narrowest and widest age ranges spanned from 45 to 64 years[4] and from 19 to 94 years.[32] None of these reports,[3–5,31–39] dichotomized according to age or presented subgroup analyses in seniors. The blood pressure component used for adjustment in the analyses was either systolic[3–5] or diastolic[3] or mean arterial pressure[33,36,37] or hypertension status,[31,32,34,38,39] whereas no study considered pulse pressure. By and large, the findings showed that retinal arterial narrowing was associated with[31,33,34] or predicted[5,39] decline in eGFR, micro- or macro-albuminuria,[31,34] lacunar stroke,[32,33,36,38] coronary heart disease[3,35] or congestive heart failure.[37] The lower boundary of the upper CRAE quantile used as the reference group ranged from 146.5[31] to 190 μm.[3] The upper boundary of the lowest quantile used as the group at risk, ranged from 128.5[31] to 160.0 μm[3] and encompassed the 150-μm threshold proposed in the current study. Limiting the reviewed literature to the four studies[5,31,33,39] with focus on renal end points, the upper boundary of the lowest quantile ranged from 128.5[31] to 144.0 μm.[39] However, mean age in these three studies ranged from 49[33] to ~60 years.[5,39]

We attempted to derive a CRAE threshold corresponding with pulse pressure levels ranging from 63 to 73 mm Hg. In these categorical analyses, we used models that at each 1-mm Hg step included a design variable (0, 1), contrasting participants with a pulse pressure above and below the successive pulse pressure thresholds. Recategorizing participants explains why there was no gradual drop off in Δ standardized CRAE with increasing pulse pressure and why the difference in CRAE was significant when participants were dichotomized based on pulse pressure thresholds ranging from 69 to 73 mm Hg (Figure 2).

One of our key findings was the positive relation between CRAE and eGFR. Several lines of evidence support the viewpoint that retinal arteriolar narrowing may coexists with small-vessel damage in the kidney. Indeed, in diabetic patients narrower CRAE is morphologically related to extracellular matrix accumulation in kidney biopsies,[40] a process that leads to diabetic nephropathy.[41] Matrix deposition in the mesangium reduces glomerular filtration surface density and decreases eGFR.[42] Finally, endothelial dysfunction might be a common hallmark of retinopathy[43] and chronic kidney disease.[44]

Our current observation that a CRAE threshold differentiates normal from slightly to moderately decreased eGFR is in line with pathophysiological concepts. The retina is an extension of the brain. Unique features of the cerebrovascular and renal vascular bed are that they are perfused at high-volume flow throughout systole and diastole.[1] Their vascular resistance is very low. Both organs throb with each beat of the heart, and their venous efflux retains pulsatility transmitted through the arteriolar, capillary and venular network.[1] It comes therefore as no surprise that a CRAE threshold derived from an index of the pulsatile component of blood pressure helps distinguishing normal from decreased eGFR. In line with this concept and our current findings is that several studies showed that the diameter of the retinal arterioles and venules change during the cardiac cycle.[2,45–47] Retinal arteriolar and venular diameter peak in mid-systole and early diastole, respectively, the maximal diameter changes averaging 3.5% and 4.8%, respectively.[2] We did not account for pulsatility of the retinal microvessels in our present study. If anything, this would bias the proposed threshold to the average between systolic and diastolic retinal arteriolar diameter. In addition, none of the aforementioned studies,[3–5,14,15,31–39] applied electrocardiographic gating to account for pulsatility.

The present study must be interpreted within the context of some limitations. First, our study had relatively small sample size and its

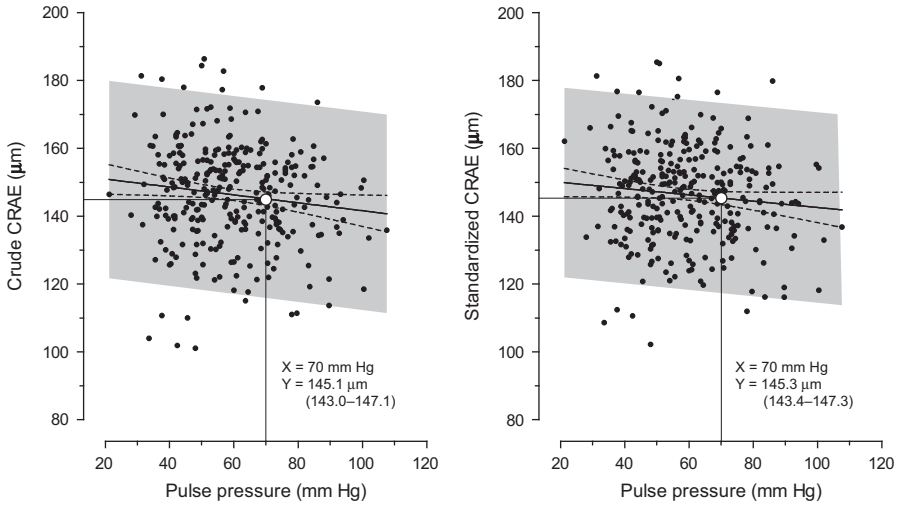

Figure 3 Derivation of thresholds for the crude and standardized central retinal arteriolar equivalent (CRAE) by the regression method. The regression line for the plotted data is drawn with 95% confidence interval (curved lines) and the 95% prediction interval for individual data points (shaded area).

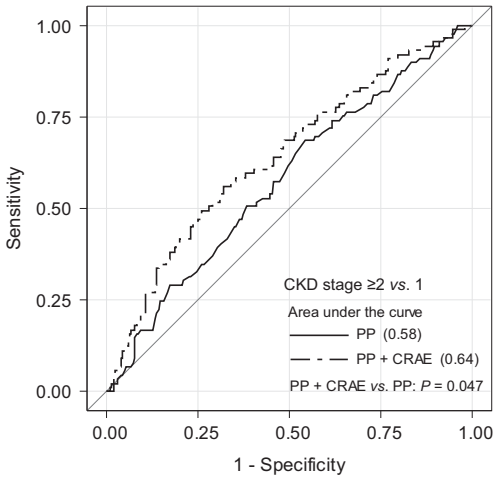

Figure 4 Receiver-operating characteristics curves to identify participants with chronic kidney disease (CKD) stage ⩾2 from stage 1. The *P*-value indicates the significance of comparison between the area under the curve of pulse pressure (PP) alone *vs.* combined with central retinal arteriolar equivalent (CRAE).

design was cross-sectional. The currently proposed CRAE threshold therefore needs refinement in a larger cohort with prospectively recorded micro- and macrovascular end points. Second, the 150- μm threshold was derived in a White population. In multivariable analyses of the Multi-Ethnic Study of Atherosclerosis stratified for ethnicity,[5] narrower CRAE was associated with a higher risk of developing CKD stage 3 in Whites, but not African Americans, Chinese or Hispanics. Confirmation of our findings in other ethnic groups is therefore required. On the other hand, we found no ethnic differences between Chinese and Whites in the association of the sublingual microcirculatory characteristics with established cardiovascular risk factors.[48] Finally, as in other population studies, we did not account for vasomotion. However, we standardized the conditions under which our participants were examined and asked them to refrain from heavy exercise, smoking, drinking alcohol or caffeine-containing beverages for at least 3 h before the examination.

In conclusion, renal microcirculatory function as captured by eGFR or CKD stage declines in parallel with retinal arteriolar narrowing. CRAE narrows and eGFR decreases in the presence of higher pulse pressure. In addition, in seniors, a 150-μm CRAE threshold identifies early CKD defined as an eGFR below 90 ml min^{-1} per 1.73 m^2. These observations demarcate lines for future research. First, longitudinal studies in seniors recruited from populations or patient cohorts should confirm the association between renal and retinal microvascular phenotypes and the utility of the 150-μm CRAE threshold in stratifying for renal risk. Second, further studies comparing non-gated and electrocardiographic-gated retinal photographs should test whether accounting for pulsatility through the cardiac cycle might improve repeatability of the measurement of the retinal microvascular diameters and enhance the potential of picking up associations with renal function. In the meantime, based on our current findings, clinicians might refer older patients with a pulse pressure of 70 mm Hg or more for retinal imaging and carefully follow-up eGFR in those with CRAE below 150 μm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The European Union (HEALTH-2011.2.4.2-2-EU-MASCARA, HEALTH-F7-305507 HOMAGE and the European Research Council Advanced Researcher Grant-2011-294713-EPLORE) and the Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Ministry of the Flemish Community, Brussels, Belgium (G.0881.13 and G.088013) currently support the Studies Coordinating Centre in Leuven. We gratefully acknowledge the contribution of the nurses working at the examination centre (Linda Custers, Marie-Jeanne Jehoul, Daisy Thijs and Hanne Truyens) and the clerical staff at the Studies Coordinating Centre (Annick De Soete and Renilde Wolfs).

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