EXTENDED METHODS

Participants and Design

Data for this analysis was obtained from The Walkerton Health Study (2002-2008), a prospective cohort study designed to evaluate the long-term health sequelae following exposure to bacterially contaminated water.[1] The study sample has previously been shown to be representative of the target population, with a slight over-representation of women and a slight under-representation of the very elderly.[2] Ethics approval was obtained from the University of Western Ontario's Research Ethics Board for Health Sciences. Of 3371 participants ≥18 years, we excluded 797, including those who developed haemolytic uremic syndrome as a result of the outbreak (n=2) and those with fewer than three annual eGFR measurements (n=791) or missing baseline data for random urine protein (n=4). Those excluded were more likely to be male (48% vs. 40%, $P<.001$), younger (difference: 4.6 years, $P<.001$), have a lower BMI (difference: 0.66 kg/m^2, p=0.005), a higher mean eGFR (difference: 3.53 $ml/min/1.73m^2$, $P<.001$), and were less likely to have a family history of hypertension (36% vs. 45%, $P<.001$) or diabetes (22% vs. 28%, $P<.001$). There were no significant differences in baseline blood pressure or hypertension, history of CVD, or family history of kidney disease.

Measures

Participants completed a baseline assessment that included questions on risk factors, family history, and doctor-diagnosed health conditions (following the format of the US Third National Health and Nutrition Examination Survey). We measured height, weight, and blood pressure using standardized protocols, and blood and urine samples were collected annually.[1] We used a urine dipstick for protein (Bayer 8SG Multistix) to measure protein from a random spot

urine sample, and the IMAGE Beckman Coulter immunoassay (Fullerton, CA, USA) to measure the albumin:creatinine ratio (ACR). Serum creatinine was measured by the modified kinetic method of JAFFE using a Vitros 950 autoanalyzer, with an interassay coefficient of 4% (reference normal range: 59-117 umol/l for males and 51-95 umol/l for females). In 2002, we calibrated 144 creatinine samples to the Cleveland Clinic and found our samples to be 0.03 mg/dl higher than the reference sample; thus, a slight underestimation of average eGFR and overestimation of annual change is expected, particularly in those with a GFR >90 ml/min/1.73m^2.[3] We calculated eGFR using the abbreviated MDRD equation [186 x (serum creatinine in mg/dl)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.212 if black)].[4]

To calculate the rate of change in eGFR over time, we fitted an ordinary least-squares (OLS) regression line to all eGFR measures for each participant. The slope of the regression line describes the rate of change in kidney function over time. Based on previous research, we defined rapid kidney function decline (RKFD) as both an absolute annual decline >3 mL/min/1.73m^2/yr[5] and as a percentage annual decline >5% (slope/baseline eGFR*100).[6] We defined CVD as a self-reported, doctor-diagnosed heart attack, stroke, or congestive heart failure and recorded the date of diagnosis. We defined diabetes mellitus by the current diagnostic criteria[7] based on random, fasting, or 2-hour post OGTT plasma glucose, or current medication (oral hypoglycaemic agents or insulin). We classified participants taking anti-hypertensive medication or presenting with a systolic/diastolic blood pressure ≥140/90 mmHg (or ≥130/80 mmHg in the presence of diabetes or eGFR<60 mL/min/1.73m^2)[8] as hypertensive. We examined two random urine screening tests for proteinuria obtained at baseline: the dipstick for protein (≥trace, ≥1g/L, and ≥3g/L) and albuminuria (ACR>2.0 mg/mmol if male or >2.8 mg/mmol if female).[9;10] We chose additional covariates based on their biological plausibility or

prior studies, including: male gender, obesity (body mass index $\geq$30 kg/m^2), and family history of diabetes, kidney disease or hypertension. We distinguished between recognized clinical risk factors for RKFD (age$\geq$60, CVD, diabetes, and hypertension) and screening tests for urine protein to improve prediction of RKFD.

Analysis

We used SAS 9.2 (SAS Institute, Inc. 1999. Cary, NC) for all analyses and summarized normally distributed data by the mean and standard deviation (SD) and skewed distributions by the median and interquartile range (IQR). We tested the associations between RKFD and baseline characteristics and screening tests using t-tests and chi-square tests as appropriate. We used a log-binomial model to estimate the adjusted relative risk (RR) for RKFD and tested covariates for inclusion at alpha=0.20 and interactions among clinical risk factors, as well as interactions between clinical risk factors and baseline eGFR<60 at alpha=0.05. We estimated the adjusted RR for RKFD at three separate cut-points for dipstick protein ($\geq$trace, $\geq$1g/L, $\geq$3g/L) and albuminuria at baseline by adding them separately to the clinical risk model. All models were run with and without outliers, and with and without adjustment for baseline eGFR. We compared the fit of models with and without adjustment for continuous or categories of baseline GFR by examination of the fit statistics and residual distribution. Adjusting for baseline eGFR had minimal impact on parameter estimates and standard errors, except for a slight increase in the strength of association with age (1.73 to 1.91) and a concomitant increase in the standard error (0.26 to 0.32); therefore we report models without adjustment for baseline eGFR as this more closely resembles the clinical situation when screening for RKFD prior to serial assessment of eGFR. We verified the assumption of persistent linear growth by graphical analysis of the

smooth nonparametric trajectories compared to the fitted OLS trajectories stratified by eGFR category at baseline and by examination of the distribution of the standard error of the OLS estimates of change. We assessed the diagnostic utility of thresholds for dipstick protein and albuminuria to identify RKFD, both overall and within high-risk subgroups (definitions in **Appendix I**).

Reference List

1. Clark W, Sontrop J, Macnab J, Salvadori M, Moist L, Suri R, Garg A: Long-term risk for hypertension, renal impairment and cardiovascular disease after gastroenteritis from drinking water contaminated with *E.coli* O157:H7:Findings from a prospective cohort study. *British Medical Journal* 341:c6020, 2010

2. Garg AX, Macnab J, Clark W, Ray JG, Marshall JK, Suri RS, Devereaux PJ, Haynes B: Long-term health sequelae following *E.coli* and *Campylobacter* contamination of municipal water. Population sampling and assessing non-participation biases. *Can J Public Health* 96:125-130, 2005

3. Clark WF, Macnab JJ, Chen SJ, Suri R, Moist L, Garg AX: Evaluation of GFR estimating equations in the general community: implications for screening. *Clin J Am Soc Nephrol* 1:787-795, 2006

4. Levey AS, Greene T, Kusek JW, Beck GJ: A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol* 11:155A, 2000

5. Rifkin DE, Shlipak MG, Katz R, Fried LF, Siscovick D, Chonchol M, Newman AB, Sarnak MJ: Rapid Kidney Function Decline and Mortality Risk in Older Adults. *Arch Intern Med* 168:2212-2218, 2008

6. 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* 20:2617-2624, 2009

7. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 26 Suppl 1:S5-20, 2003

8. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr., Roccella EJ, the National High Blood Pressure Education Program Coordinating Committee: Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 42:1206-1252, 2003

9. Whaley-Connell AT, Sowers JR, Stevens LA, McFarlane SI, Shlipak MG, Norris KC, Chen SC, Qiu Y, Wang C, Li S, Vassalotti JA, Collins AJ: CKD in the United States: Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Am J Kidney Dis* 51:S13-S20, 2008

10. Vassalotti JA, Stevens LA, Levey AS: Testing for chronic kidney disease: a position statement from the National Kidney Foundation. *Am J Kidney Dis* 50:169-180, 2007

Appendix I

This Appendix contains the results of several sensitivity analyses that test the robustness of our main analysis. Specifically, we evaluated whether the imprecision of the MDRD GFR estimating equation at higher levels of GFR affected our results or conclusions. In brief, we first examined two definitions of kidney function decline (KFD), percentage change vs. absolute change (summarized in **A1** below) and chose to report results for the definition least susceptible to error due to the imprecision of the MDRD at higher levels of GFR. We also conducted several sensitivity analyses to test the robustness of our findings and account for the higher variability in those with eGFR>90 (see **A2 and A3** below). We excluded those with eGFR>90 ml/min/1.73 m^2 at their first or final assessment, and we also excluded participants with highly variable rates of kidney function decline. As shown in **Tables A2 and A3**, dipstick proteinuria remained a stronger predictor of RKFD than albuminuria, regardless of how RKFD was defined, or if we excluded those with eGFR>90 or those with highly variable rates of KFD.

A1. Percentage decline (KFD>5%) vs. absolute decline (KFD>3 mL/min/1.73 m^2/yr).

To calculate the rate of change in eGFR over time, we fitted an ordinary least-squares regression line to all eGFR measures for each participant. The slope of the regression line describes the rate of change in kidney function over time. Based on previous research, we defined rapid kidney function decline (RKFD) as both a percentage annual decline (KFD>5%)[1] and an absolute annual decline (KFD>3 ml/min/1.73 m^2/yr).[2]

The imprecision of the MDRD equation above 90 ml/min/1.73m^2 resulted in the distribution of the absolute annual change being strongly skewed to the right and more susceptible to highly variable rates of annual decline in those with baseline eGFR ≥90. As a result, 15.6% had KFD>3 ml/min/1.73m^2/yr compared to only 8.5% with KFD>5%. The distribution of percentage annual change was more symmetrically balanced around the median with only 3 outliers (all 3 had baseline eGFR<60).

By definition, KFD>5% places greater importance on declining kidney function among those with less renal reserve. For instance, compared to those with KFD>3 ml/min/1.73m^2/yr, KFD>5% is more common in those with an eGFR<60 (13% vs. 19%) and is equally distributed (~ 8%) across GFR categories above 60 ml/min/1.73m^2 (see **Figure 1** in manuscript). In contrast, those with eGFR above 90 ml/min/1.73m^2 were nearly three times as likely to be identified as having KFD>3 ml/min/1.73m^2/yr vs. KFD>5% (23% vs. 8%). At higher levels of eGFR, the clinical meaning of KFD>3 ml/min/1.73m^2/yr is less clear, and is more likely to result from measurement error or age-related decline rather than renal pathology. For instance, 65% of those with KFD>5% progressed to a new CKD stage, compared to 60% of those with KFD>3 ml/min/1.73m^2/yr. Of those who progressed, twice as many with an initial eGFR above 90 were flagged by KFD>3 ml/min/1.73m^2/yr vs. KFD>5% (**Table A1**). Most importantly, KFD>5% was a stronger indicator of risk than absolute change. The risk for future cardiovascular disease was substantially greater in those with KFD>5% (RR=2.7; p=0.004) compared to those with KFD>3ml/min/1.73m^2 (RR=1.4; p=0.35). Because CVD was not the

primary outcome in the Walkerton Health Study, assessment was from self-reported doctor-diagnosed health conditions. However, the sensitivity and specificity of self-reported CVD in the literature is good, ranging from 66-90%[3-5] and 98-99%,[3;5] respectively. Given that participants were unaware of their kidney function decline classification, differential misclassification is unlikely, and as such, the measurement error introduced by self-report should lead to an attenuation of the observed effects.

Thus, in a general population sample, KFD>3 ml/min/1.73m^2/yr appears more likely to flag individuals whose kidney function is declining due to aging (or from measurement error). Percentage annual change places more significance on small changes in the presence of reduced renal reserve and less significance on small changes within the normal range of kidney function. Since KFD>5% demonstrated greater clinical utility and was less susceptible to imprecision of the MDRD at eGFRs above 90, our main results reflect the analyses of KFD>5%; however, a summary of the results for absolute annual decline are provided below in **Tables A2 and A3.** Importantly, our main conclusions remain the same whether RKDF is defined as KFD>5% or KFD>3ml/min/1.73m^2. As shown in **Tables R2 and R3**, dipstick protein consistently outperforms albuminuria as a predictor of RKFD, regardless of which indicator is used.

A2. Effect of excluding those with eGFR>90 ml/min/1.73m^2

To assess the impact of the imprecision of the MDRD equation at eGFR above 90 ml/min/1.73m^2 we performed two sensitivity analyses that alternately excluded participants who's first or final eGFR values were above 90 ml/min/1.73m^2. These exclusions resulted in similar or stronger associations between the screening tests and RKFD; however, the overall pattern remained the same (**Tables A2 and A3**, below). As well, restricting the sample to those with a baseline eGFR <90 ml/min/1.73m^2 had little impact on the measures of diagnostic utility (see **Table A4** below).

A3. Effect of excluding those with extreme variability

Although repeated testing adjusts for measurement error from random variation, measurement error arising from the imprecision of the MDRD equation at eGFR above 90 ml/min/1.73m^2 could result in excess variability in the assessment of renal decline; therefore, we performed a sensitivity analysis that excluded those with extreme variability in renal decline. Excluding cases in which the standard error of the slope for renal decline was greater than the 90th percentile produced similar or stronger associations between the screening tests and risk for RKFD (see **Tables A2 and A3**). Again, dipstick protein remained a stronger predictor of RKFD compared to albuminuria, particularly for RKFD>5%.

Table A1. Progression to a new CKD Stage during follow up.

eGFR at baseline	N	Number who progress to a new CKD stage	Percent flagged by KFD>5%	Percent flagged by KFD>3 ml/min/1.73m^2/yr
<30	10	5	100%	20%
30-59	188	8	75%	63%
60-89	1479	222	32%	41%
>=90	897	354	17%	41%

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; KFD, kidney function decline.

Table A2. Summary of sensitivity analyses for rapid kidney function decline defined as a percentage decline >5% from baseline

Model		Sample size	Relative Risk[a] (95% Confidence Interval)			
				Dipstick Protein		
			Albuminuria[b]	Trace or above	≥1 g/L	≥3 g/L
1	Percentage Annual Decline >5%	2574	1.5 (1.1-2.2)	2.2 (1.5-3.2)	3.0 (1.9-4.7)	3.3 (2.0-5.5)
2	Exclude if first eGFR ≥90 ml/min/1.73m^2	1677	1.8 (1.2-2.7)	2.4 (1.6-3.7)	3.2 (1.9-5.4)	3.3 (1.8-5.8)
3	Exclude if final eGFR ≥90 ml/min/1.73m^2	1873	1.5 (1.1-2.2)	2.1 (1.5-3.1)	2.7 (1.7-4.3)	2.8 (1.7-4.7)
4	Exclude if SE of slope ≥90th percentile	2315	1.5 (1.1-2.3)	2.3 (1.5-3.4)	3.0 (1.8-4.8)	3.0 (1.7-5.2)

Abbreviations: eGFR, estimated glomerular filtration rate; KFD, kidney function decline; SE, standard error.

[a] Relative risks were estimated from a log-binomial regression adjusted for age, hypertension, diabetes, and cardiovascular disease.

[b] Random albumin to creatinine ratio (ACR) >2.0 mg/mmol (17 mg/g) if male or ACR>2.8 mg/mmol (25 mg/g) if female).[6;7]

Table A3. Sensitivity analyses for rapid kidney function decline defined as annual decline >3 ml/min/1.73m^2

Model		Sample size	Relative Risk[a] (95% Confidence Interval)			
				Dipstick Protein		
			Albuminuria[b]	Trace or above	≥1 g/L	≥3 g/L
5	KFD> 3 ml/min/1.73m^2	2574	1.4 (1.0-1.9)	1.6 (1.1-2.2)	1.7 (1.0-2.9)	2.1 (1.2-3.7)
6	Exclude if first eGFR ≥90 ml/min/1.73m^2	1677	1.4 (1.0-2.1)	1.8 (1.2-2.8)	2.4 (1.4-4.1)	2.7 (1.5-4.8)
7	Exclude if final eGFR ≥90 ml/min/1.73m^2	1873	1.4 (1.0-1.9)	1.5 (1.1-2.2)	1.8 (1.2-2.9)	2.1 (1.3-3.5)
8	Exclude if SE of slope ≥90th percentile	2315	1.5 (1.0-2.0)	1.6 (1.1-2.3)	1.9 (1.2-3.1)	2.2 (1.3-3.8)

Abbreviations: eGFR, estimated glomerular filtration rate; KFD, kidney function decline; SE, standard error.

[a] Relative risks were estimated from a log-binomial regression adjusted for age, hypertension, diabetes, and cardiovascular disease.

[b] Random albumin to creatinine ratio (ACR) >2.0 mg/mmol (17 mg/g) if male or ACR>2.8 mg/mmol (25 mg/g) if female).[6;7]

Table A4. Diagnostic utility of dipstick proteinuria and albuminuria at baseline to identify patients with rapid kidney function decline: Compares overall results to those with eGFR<90 ml/min/1.73m^2

	n	%	Pretest prob-ability of RKFD	Screen positive %	False positive rate	LR+ /LR-	Posttest prob-ability of RKFD	NNTS	NNTF
OVERALL	2574	100%	0.09						
Albuminuria[a]	253			9.8	8.9	3.2	0.20	10.2	5
Dipstick protein									
≥Trace	145			5.6	4.7	3.7	0.23	17.8	4.3
≥ 1 g/L	63			2.5	1.7	7.2	0.38	40.9	2.6
≥ 3 g/L	42			1.6	1.1	7.8	0.41	61.3	2.5
First GFR < 90									
OVERALL	1677	1	0.09						
Albuminuria[a]	178			10.6	8.9	4.2	0.24	9.4	4.2
Dipstick protein									
≥Trace	100			6.0	4.8	4.6	0.27	16.8	3.7
≥ 1 g/L	46			2.7	1.7	9.2	0.43	36.5	2.3
≥ 3 g/L	32			1.9	1.2	9.0	0.44	52.4	2.3

Abbreviations (definitions): LR+/LR-, ratio of positive to negative likelihood ratios; NNTF, number of patients needed to follow with serial serum creatinine measurements to identify one case of RKFD (the inverse of the prevalence of RKFD among screen positive); NNTS, number of patients needed to screen to identify one positive result (the inverse of the prevalence of a positive screening result); RKFD, rapid kidney function decline (% annual eGFR decline >5%).

[a]Random albumin to creatinine ratio (ACR) >2.0 mg/mmol (17 mg/g) if male or ACR>2.8 mg/mmol (25 mg/g) if female).[25;26]

Reference List

1. 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* 20:2617-2624, 2009

2. Rifkin DE, Shlipak MG, Katz R, Fried LF, Siscovick D, Chonchol M, Newman AB, Sarnak MJ: Rapid Kidney Function Decline and Mortality Risk in Older Adults. *Arch Intern Med* 168:2212-2218, 2008

3. Engstad T, Bonaa KH, Viitanen M: Validity of Self-Reported Stroke : The Tromso Study. *Stroke* 31:1602-1607, 2000

4. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, Hennekens CH, Speizer FE: Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol* 123:894-900, 1986

5. Okura Y, Urban LH, Mahoney DW, Jacobsen SJ, Rodeheffer RJ: Agreement between self-report questionnaires and medical record data was substantial for diabetes, hypertension, myocardial infarction and stroke but not for heart failure. *J Clin Epidemiol* 57:1096-1103, 2004

6. Whaley-Connell AT, Sowers JR, Stevens LA, McFarlane SI, Shlipak MG, Norris KC, Chen SC, Qiu Y, Wang C, Li S, Vassalotti JA, Collins AJ: CKD in the United States: Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES) 1999-2004. *Am J Kidney Dis* 51:S13-S20, 2008

7. Vassalotti JA, Stevens LA, Levey AS: Testing for chronic kidney disease: a position statement from the National Kidney Foundation. *Am J Kidney Dis* 50:169-180, 2007

APPENDIX II

Measures of diagnostic utility: Formulae and definitions

		Annual % Renal decline		
		> 5%*	≤ 5%	Total
Screening test	Positive	a	b	a+b
	Negative	c	d	c+d
	Total	a+c	b+d	a+b+c+d

*Rapid renal decline (RRD)

Term	Formula	Definition
Percent Agreement	a+d/a+b+c+d	Percent correctly identified as RRD or non-progressive
Sensitivity	a/(a+c)	Probability of a positive test among those with RRD
Specificity	d/(b+d)	Probability of a negative test among those without RRD
Positive likelihood ratio (+LR)	Sensitivity/(1-Specificity)	Amount of certainty gained after a positive test
Negative likelihood ratio (-LR)	(1-Sensitivity)/Specificity)	Amount of certainty gained after a negative test
Pre-test probability of RRD	(a+c)/(a+b+c+d)	Prevalence of disease in population
Pre-test odds	Prevalence/(1-Prevalence)	Odds of disease before prior to screening test
Post-test odds		
• **Positive test**	Pre-test odds x +LR	Odds of RRD after a positive test
• **Negative test**	Pre-test odds x –LR	Odds of RRD after a negative test
Post-test probability		
• **Positive test**	Post-test odds/ (post-test odds +1)	Probability of disease after a positive test
• **Negative test**	Post-test odds/ (post-test odds +1)	Probability of disease after a negative test
Number needed to screen	a+b+c+d(a+b)	Number needed to screen to identify one case with proteinuria
Number needed to follow	(a+b)/a	Number needed to follow after a positive test to identify one case with RRD