Supplemental Material

Full Methods

Study population

We performed a cross-sectional cohort study in subjects over 50 years old with CKD stages 3 or 4, defined as eGFR between 15 and 60 ml/min/1.73m^2. Exclusion criteria comprised: any chronic inflammatory disease, gout, diabetes, history of a cardiovascular event, uncontrolled hypertension, proteinuria > 1 gram/liter, overt lipid disorders and use of anti-inflammatory drugs. Because of ethical constraints relating to radiation exposure, for the imaging studies, healthy controls were selected from a contemporaneous study using identical imaging protocols and performed on the same scanner. The age limit of >50 years was chosen on the same grounds. For the ex vivo monocyte studies, healthy controls matched on age and sex were included on the same study days as the CKD patients. The study protocol was approved by the Institutional Review Board of the Academic Medical Center in Amsterdam, The Netherlands. Written informed consent was obtained from each participant.

Baseline data collection

CV-risk factors, medical history and family history, as well as medication use were assessed with a questionnaire. Physical examination, including weight, height and brachial artery blood pressure measurement using an oscillometric blood pressure device. was performed. Lipid levels, CRP, creatinine, urea, sodium, potassium, phosphate, uric acid and albumin were assessed in fasting plasma and creatinine, urea, protein, sodium and potassium in 24 hour urine, all using standard laboratory procedures. White blood cell count (WBC) and differentiation were determined with automated cell counters. eGFR was calculated using the 2009 CKD-EPI equation (www.kidney.org/professionals/kdoqi/gfr_calculator).[1]

^{18}F-FDG PET/CT imaging

^{18}F-FDG PET/CT imaging was performed on a PET/CT scanner (Philips, Best, the Netherlands) as previously described.[2] Subjects fasted for at least 6 hours prior of infusion of 100 MBq ^{18}F-FDG. 90 minutes after FDG administration, pet imaging was initiated with a low dose (40 mAs), non-contrast enhanced CT for attenuation correction and anatomic co-registration (slice thickness 3 mm), The images are acquired from the internal auditory meatus to the diaphragm, resulting in images of both carotids and the aorta. Arterial FDG uptake was quantified by drawing a region of interest around each artery on 5 slices of the co-registered transaxial images. Standardized uptake values (SUV) were averaged for each artery

(ascending aorta and both carotids), and divided by the average venous background activity (SUV_{mean}) to obtain the target-to-background-ratio (TBR).[2] The SUV is the decay-corrected tissue concentration of FDG in kBq/ml, adjusted for the injected dose. For the carotid arteries, the vessel with the highest uptake was denominated the Index vessel and used for further analyses.

Additional analyses in hypertensive subjects

Hypertensive subjects were selected from an existing cohort of patients at increased CVD risk (Framingham risk score >10%), used in a study to provide reference values for ^{18}F-FDG PET/CT imaging (Van der Valk, JACC, in press). All subjects provided written informed consent. ^{18}F-FDG PET/CT imaging was performed at the same scanner and using the same protocol as the CKD cohort, in >6 hour fasting subjects, who received 200 MBq of ^{18}F-FDG (5.5 mCi) prior to imaging. Image analyses was performed identical to the analyses performed in the CKD cohort.

Coronary calcium scores

The CT scans were also used to determine an adjusted Agatston score. The measurement equals the original score (In a manually set volume of interest, all pixels with an intensity higher than 130 HU were selected). Connected areas of these thresholded pixels were constructed. All areas smaller than 1 mm^2 were excluded. The score was determined by combining all selected connected areas with a weight. The weight was determined by the highest intensity value of a pixel in the connected areas: 1 for 130–199 HU, 2 for 200–299 HU, 3 for 300–399 HU, and 4 for 400 HU and greater. Because of the difference in slice thickness of the images in his study (5 mm) compared to default Agatston score images (3mm), the sum score was multiplied with 5/3

Flow cytometry

Red blood cells were lysed with RBC lysis buffer (Affymetrix, eBioscience). Leukocytes were incubated with fluorchrome labelled antibodies (supplemental table 1) for 15 minutes and washed with saline. Samples were analysed on a BD FACS Canto II flow cytometer (Becton, Dickinson, Fanklin Lakes, NJ). Monocytes were classified according to HLA-DR, CD14 and CD16 expression.[3] Subsequently, surface markers involved in monocyte chemotaxis were assessed (supplemental table 1 for all used markers). Samples were analyzed using FlowJo software (version 7.6.5.). Delta median fluorescence intensity (MFI) was obtained by subtracting isotype MFI from the MFI of the marker in corresponding color.

Trans-endothelial migration

To functionally assess adhesive and migratory capacity, a trans-endothelial migration (TEM) assay was performed as described previously.[4] Primary human arterial endothelial cells (HAEC, Lonza, Baltimore, MD), cultured to confluence, were stimulated with TNF-α (10 ng/ml) overnight. CD14 bead (Sigma Alderich) isolated monocytes were added at a concentration of $1*10^6$ cells/ml for 30 min at 37°C, 5% CO_2 and then fixed with 3.7% formaldehyde (Sigma-Aldrich, Zwijndrecht, the Netherlands), experiments were performed in duplicate. Multiple images were recorded with a Zeiss Axiovert 200 microscope (Plan-apochromat 10x/0.45 M27 Zeiss-objective; Carl Zeiss Inc., Jena, Germany). Adhered (bright morphology) and transmigrated monocytes (dark morphology) were quantified using the cell counter plugin (http://rsbweb.nih.gov/ij/plugins/cell-counter.html) in the Image-J software (http://rsb.info.nih.gov/nih-image/).

References

1. Levey AS, Stevens LA, Schmid CH, Zhang YL, 3rd AFC, Feldman HI, Kusek JW, Eggers P, Lente F Van, Greene T, Coresh J, Collaboration) C-E (Chronic KDE: A new equation to estimate glomerular filtration rate. *Ann. Intern. Med.* 150: 604–612, 2009

2. Rudd JHF, Myers KS, Bansilal S, Machac J, Pinto CA, Tong C, Rafique A, Hargeaves R, Farkouh M, Fuster V, Fayad Z a: Atherosclerosis inflammation imaging with 18F-FDG PET: carotid, iliac, and femoral uptake reproducibility, quantification methods, and recommendations. *J. Nucl. Med.* 49: 871–8, 2008

3. Abeles RD, McPhail MJ, Sowter D, Antoniades CG, Vergis N, Vijay GKM, Xystrakis E, Khamri W, Shawcross DL, Ma Y, Wendon JA, Vergani D: CD14, CD16 and HLA-DR reliably identifies human monocytes and their subsets in the context of pathologically reduced HLA-DR expression by CD14hi/CD16neg monocytes: Expansion of CD14hi/CD16pos and contraction of CD14lo/CD16pos monocytes in acute liver fail. *Cytom. Part A* 81 A: 823–834, 2012

4. Van Der Valk FM, Kroon J, Potters W V., Thurlings RM, Bennink RJ, Verberne HJ, Nederveen AJ, Nieuwdorp M, Mulder WJM, Fayad ZA, Van Buul JD, Stroes ESG: In vivo imaging of enhanced leukocyte accumulation in atherosclerotic lejas. *J. Am. Coll. Cardiol.* 64: 1019–1029, 2014

Supplemental table S1: Markers used for flow cytometry

Surface marker	Color	Company
CD14	PE-Cy7	BD
CD16	APC-Cy7	BD
HLA-DR	PerCpCy5.5	BD
CD192 (CCR2)	APC	BD
CCR7	PE	Biolegend
CCR5	FITC	BD

PE indicates phycoerythrin , Cy; CyChrome, APC; allophycocyanin, PerCP; peridinin-chlorophyll-protein *FITC indicates Fluorescein isothiocyanate*

Supplemental table S2 linear regression analysis with Aortic TBR_{max} as the dependent variable

Characteristic	r	P value
BMI	-0.037	0.905
Current smoking	-0.247	0.416
Statin use	0.053	0.846

Data are Pearson's correlation coefficient (r). *BMI* indicates body mass index

Supplemental Table S3: Univariate and multivariate linear regression analysis with Aortic TBR_{max} as the dependent variable

Characteristic	Unadjusted analyses		Adjusted analyses[α]	
	β (95% CI)	P value	β (95% CI)	P value
PTH	-0.164(-0.178-0.107)	0.593	-0.301(-0.255-0.124)	0.451
Calcium	-0.182(-6.101-3.445)	0.553	-0.133(-7.231-5.120)	0.704
Phosphate	0.381(-1.419-6.050)	0.200	0.529(-2.096-8.535)	0.200
Calcium*Phosphate	0.284(-0.759-1.978)	0.348	0.362(-1.067-2.632)	0.395
Uric Acid	0.081(-7.540-9.624)	0.793	0.265(-8.988-15.826)	0.541

Data are standardized coefficient (β) with 95% confidence intervals (CI). *PTH* indicates parathyroid hormone.

Supplemental table S4: Clinical characteristics of CKD patients compared to Hypertensive subjects

Characteristic	CKD (n=14)	Hypertensive (n=8)	P value
Sex, male/female	7/7	8/0	0.030
Age, y	60.8±8	61.6±5	0.792
Body mass index, kg/m^2	25.2±4	28.9±3	0.028
Smoking, yes/no	3/11	0/8	0.159
SBP, mm Hg	135±18	144±8	0.182
DPB, mm Hg	80±8	90±8	0.015
MAP, mm Hg	99±10	108±4	0.010
Creatinine, umol/L	183[123-197]	80[78-89]	<0.001
eGFR (CKD-EPI), ml/min/1.73 m^2	37±12	86±6	<0.001
Total cholesterol, mmol/L	5.7±1.3	6.0±2.5	0.750
LDL cholesterol, mmol/L	3.5±1.0	4.1±2.2	0.371
HDL cholesterol, mmol/L	1.4±0.31	1.2±0.27	0.156
Triglycerides, mmol/L	1.34[0.98-1.81]	1.5[0.81-1.63]	0.525
CRP, mg/dl	2.2[0.7-3.8]	1.3[1.1-2.9]	0.779
WBC, 10E9/L	5.3±1.6	5.5±1.2	0.750
- Lymfocytes	1.5±0.5	1.9±0.6	0.075
- Neutrofils	3.2±1.4	2.7±0.6	0.315
- Monocytes	0.4±0.1	0.5±0.1	0.201

Values are n, mean ± SD or median [IQR,] for skewed data. *SBP* indicates systolic blood pressure; *DBP* diastolic blood pressure; *MAP* mean arterial pressure; *eGFR* estimated glomerular filtration rate; *LDL* low density lipoprotein; *HDL* high density lipoprotein; *CRP* c reactive protein; *WBC* white blood cell count.

Supplemental table S5: TBR in CKD patients compared to hypertensive subjects

	CKD (n=14)	Hypertensive (n=8)	Adjusted p-value
Aorta	3.1±0.7	2.8±0.5	0.043
Aorta$_{mds}$	3.2±0.7	2.9±0.5	0.048
Carotid	2.5±0.7	2.1±0.4	0.179
Carotid$_{mds}$	2.6±0.7	2.2±0.5	0.139

Data are mean± SD. *CKD* chronic kidney disease; *mds* Mean arterial pressure. p is adjusted for gender and BMI

Supplemental Table S6: Clinical characteristics of CKD patients and healthy controls – ex vivo monocyte studies

Characteristic	Control (n=14)	CKD (n=14)	P value
Sex, male/female	7/7	7/7	1.000
Age, y	59.4±6	58.6±5	0.713
Body mass index, kg/m^2	25.6±3	25.2±4	0.735
Smoking, yes/no	1/13	3/11	0.280
SBP, mm Hg	133±11	135±18	0.785
DPB, mm Hg	86±10	80±8	0.079
eGFR (CKD-EPI), ml/min/1.73 m^2	87±13	37±12	<0.001
Creatinine, umol/L	75[68-83]	182[124-197]	<0.001
Total cholesterol, mmol/L	5.5±1.0	5.7±1.3	0.642
LDL cholesterol, mmol/L	3.3±0.78	3.5±1.0	0.628
HDL cholesterol, mmol/L	1.6±0.44	1.4±0.31	0.188
Triglycerides, mmol/L	0.77[0.60-1.20]	1.36[1.02-1.83]	0.023
CRP, mg/dl	1.4[0.8-1.7]	2.4[0.7-4.1]	0.310
WBC, 10E9/L	5.5±0.8	5.3±1.6	0.728
- Lymfocytes	1.8±0.6	1.5±0.5	0.209
- Neutrofils	3.1±0.7	3.2±1.4	0.873
- Monocytes	0.4±0.09	0.4±0.1	0.934

Values are n, mean ± SD or median [IQR,] for skewed data. *SBP* indicates systolic blood pressure; *DBP* diastolic blood pressure; *eGFR* estimated glomerular filtration rate; *LDL* low density lipoprotein; *HDL* high density lipoprotein; *CRP* c reactive protein; *WBC* white blood cell count.