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Severely increased albuminuria in patients with type 2 diabetes mellitus is associated with increased subclinical atherosclerosis in femoral arteries with Na [^{18}F]F activity as a proxy - The DETERMINE study

M. Reijrink [a,*], J.K.E. Sluiter [a], C.A. te Velde-Keyzer [b], M.H. de Borst [b], G.D. van Praagh [c], M.J.W. Greuter [d], G. Luurtsema [c], H.H. Boersma [c,e], R.A. Pol [f], J.L. Hillebrands [g], P.R. van Dijk [h], K. Hoogenberg [i], D.J. Mulder [a], R.H.J.A. Slart [c,j]

[a] University of Groningen, University Medical Center Groningen, Dept. Internal Medicine, div. Vascular Medicine, the Netherlands
[b] University of Groningen, University Medical Center Groningen, Dept. Internal Medicine, div. Nephrology, the Netherlands
[c] University of Groningen, University Medical Center Groningen, Dept. Nuclear Medicine and Molecular Imaging, the Netherlands
[d] University of Groningen, University Medical Center Groningen, Medical Imaging Center, Department of Radiology, Groningen, the Netherlands
[e] University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, the Netherlands
[f] University of Groningen, University Medical Center Groningen, Department of Vascular and Transplant Surgery, Groningen, the Netherlands
[g] University of Groningen, University Medical Center Groningen, Dept. Pathology and Medical Biology, div. Pathology, the Netherlands
[h] University of Groningen, University Medical Center Groningen, Dept. Internal Medicine, div. Endocrinology, the Netherlands
[i] Department of Internal Medicine, Martini Hospital, Groningen, the Netherlands
[j] University of Twente, Dept. of Biomedical Phototonic Imaging, Enschede, the Netherlands

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ABSTRACT

Background and aims: Sodium [^{18}F]fluoride (Na [^{18}F]F) positron emission tomography imaging allows detailed visualization of early arterial micro-calcifications. This study aims to investigate atherosclerosis manifested by micro-calcification, macro-calcification, and aortic stiffness in patients with type 2 diabetes mellitus (T2DM) with and without albuminuria and severely decreased kidney function.

Methods: A cohort was stratified in four groups (N = 10 per group), based on KDIGO categories (G1-5 A1-3). G1-2A1 non-diabetic controls (median [IQR] estimated glomerular filtration rate (eGFR) in mL/min/1.73 m^2 91 [81–104]), G1-2A1 with T2DM (eGFR 87 [84–93], and albumin-creatinin-ratio (ACR) in mg/mmol 0.35 [0.25–0.75]), G1-2A3 with T2DM (eGFR 85 [60–103], and ACR 74 [62–122], and G4A3 with T2DM (eGFR 19 [13-27] and ACR 131 [59–304]).

Results: Na [^{18}F]F femoral artery grading score differed significantly in the groups with the highest Na [^{18}F]F activity in A3 groups with T2DM (G1-2A3 with T2DM 228 [100–446] and G4A3 with T2DM 198 [113–578]) from the lowest groups of the G1-2A1 with T2DM (33 [0–93]) and in G1-2A1 non-diabetic controls (75 [0–200], $p = 0.001$). Aortic Na [^{18}F]F activity and femoral artery computed tomography (CT)-assessed macro-calcification was increased in G4A3 with T2DM compared with G1-2A1 with T2DM (47.5 [33.8–73.8] *vs.* 17.5 [8.8–27.5] ($p = 0.006$) and 291 [170–511] *vs.* 12.2 [1.41–44.3] mg ($p = 0.032$), respectively). Carotid-femoral pulse wave velocity (PWV)-assessed aortic stiffness was significantly higher in both A3 groups with T2DM compared with G1-2A1 with T2DM (11.15 and 12.35 *vs.* 8.86 m/s, respectively ($p = 0.009$)).

Conclusions: This study indicates that the presence of severely increased albuminuria in patients with T2DM is cross-sectionally associated with subclinical arterial disease in terms of micro-calcification and aortic stiffness. Additional decrease in kidney function was associated with advanced macro-calcifications.

1. Introduction

In patients with type 2 diabetes mellitus (T2DM), hyperglycaemia and glycaemic fluctuations result in premature vascular ageing, including arterial calcification, which might develop into cardiovascular diseases (CVD) [1,2]. Premature vascular ageing is even more

* Corresponding author.
E-mail address: m.reijrink@umcg.nl (M. Reijrink).

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pronounced in patients with diabetic kidney disease as a result of extracellular matrix deposition, elevated phosphate levels, uremic milieu, or a combination of these [3–5]. It is well established that decreased kidney function due to diabetic kidney disease leads to widespread calcifications [6]. Next to a decreased kidney function as marker of chronic kidney disease (CKD), the presence of albuminuria, as clinical marker of glomerular filtration impairment, is independently associated with CVD [7–9]. Moreover, kidney function and albuminuria, classified in Kidney Disease: Improving Global Outcomes (KDIGO) categories, are independent risk factors for all-cause mortality and CVD-mortality in patients with T2DM [6]. The presence of albuminuria, even with a preserved kidney function, is a well-known risk factor for the progression of kidney disease and cardiovascular outcomes [10–12]. But, whether albuminuria in patients with T2DM and a preserved kidney function could serve as early clinical sign and intervention target of subclinical, potentially reversible, atherosclerosis is still unclear.

Detection of subclinical atherosclerosis is a major challenge due to the absence of clinical CVD symptoms. These premature stages of arterial wall abnormalities, such as micro-calcifications, may allow for preventive therapy and, therefore, inhibit further development in clinically manifest CVD. One of the reversible endpoints of CVD development is aortic stiffness which can be measured with, for instance, B-mode carotid ultrasound-assessed intima-media thickness (IMT), pulse pressure measurements and pulse wave velocity (PWV) [13]. PWV provides an index of aortic stiffness and underlying atherosclerosis [14]. Next to a decreased glomerular filtration rate (GFR), increased albumin-to-creatinine ratio (ACR), reflecting albuminuria, is a significant predictor for increased PWV in patients with T2DM [15].

While advanced macro-calcifications can be easily observed with computed tomography (CT), sodium [^{18}F]fluoride (Na [^{18}F]F) molecular positron emission tomography (PET) imaging allows for detailed visualization and quantification of early active micro-calcifications in the arterial wall [16,17]. This may then allow for the selection of patients that could benefit from preventive therapy. Since it is unknown whether albuminuria is associated with increased subclinical atherosclerosis, the aim of this study is to investigate whether albuminuria coincides with harbingers of arterial disease. Non-invasive diagnostic modalities such as CT, Na [^{18}F]F PET and PWV serve as markers of arterial disease by measuring macro-calcification, micro-calcification, and aortic stiffness, respectively.

2. Patients and methods

2.1. Study design

This single center, cross-sectional, observational, case-control study was performed in compliance with the principles of the Declaration of Helsinki. All patients gave written informed consent. The study protocol was reviewed and approved by the Institutional Review Board of the University Medical Center Groningen (UMCG, Research Register number: 201800548, METc number 2018.456 [for patients with T2DM], Research Register number 202000855 [for G1-2A1 non-diabetic controls]).

2.2. Study population

Potential eligible participants with T2DM were selected from the department of Internal Medicine of the UMCG and the Martini Hospital Groningen, and from the department of Nuclear Medicine and Molecular Imaging of the UMCG. Participants from the UMCG were selected using the electronic patient health records by selecting applicable characteristics. In addition, a database with patients with T2DM from the Martini Hospital Groningen was used for screening and selection of participants. Preserved kidney function, with eGFR >60 mL/min/1.73 m^2 was expressed as G1-2 and severely decreased kidney function, with eGFR 15–29 mL/min/1.73 m^2 was expressed as G4, based on KDIGO international guidelines [18]. Also according to these guidelines, no albuminuria (KDIGO A1) was defined as ACR <3 mg/mmol, moderate albuminuria (A2) as ACR 3–30 mg/mmol and severely increased albuminuria (A3) as ACR >30 mg/mmol [18]. G1-2A1 non-diabetic controls and patients with T2DM aged between 18 and 80 years were included. Participants were age- and sex-matched for four study groups: 1) G1-2A1 non-diabetic controls, 2) G1-2A1 patients with T2DM, 3) G1-2A3 patients with T2DM, and 4) G4A3 patients with T2DM (eGFR 15–30 mL/min/1.73 m^2). Patients on renal replacement therapy were excluded to eliminate the influence of dialysis. T2DM was defined according to the criteria formulated by the American Diabetes Association [19]; fasting plasma glucose ≥7.0 mmol/L (≥126 mg/dL) and/or a random plasma glucose ≥11.1 mmol/L (200 mg/dL) and/or a hemoglobin A_{1C} (HbA_{1C}) ≥6.5% (≥48 mmol/L).

Exclusion criteria for all participants were T1DM (characterized by the World Health Organization as deficient insulin production and daily administration of insulin required), clinically significant liver disease such as (non-)alcoholic fatty liver disease or liver fibrosis, pregnancy or breastfeeding, active bone malignancy in the six months before inclusion, disorders affecting the bone metabolism (e.g. Paget's disease), uncontrolled hypertension (systolic blood pressure >200 mmHg), history of recent (in previous 12 months) CVD defined as stable coronary artery disease or acute coronary syndrome, stroke or transient ischemic attack, or peripheral artery disease. G1-2A1 non-diabetic controls were considered eligible when the above-mentioned exclusion criteria, T2DM, history of chemotherapy and metastasis were not present.

2.2.1. Clinical and laboratory assessments

The following clinical data were collected: age, sex, medical history, drug use, cardiovascular history, and smoking habits. As a marker of kidney function eGFR was calculated using the CKD-EPI formula and expressed in mL/min/1.73 m^2 [20]. Morning blood samples were obtained from all patients with T2DM after at least 6 h of overnight fasting and plasma glucose, insulin, HbA_{1C}, lipid profile (total cholesterol, HDL, LDL, triglycerides, calcium, and phosphate were measured using routine automated assays. Insulin resistance (IR) was estimated with two methods; 1) with the HOMA-IR: fasting insulin * fasting glucose/22.5 [21], and 2) with triglycerides/HDL cholesterol ratio [22].

2.3. Arterial stiffness

Pulse pressure was calculated as the difference between systolic blood pressure and diastolic blood pressure values [14]. Aortic stiffness was non-invasively assessed in patients with T2DM as SphygmoCor PWV, but not in the G1-2A1 non-diabetic controls since they were included retrospectively. The SphygmoCor PWV method was described in more detail elsewhere [23,24]. In brief, pressure waves were measured between the left carotid artery (proximal) and left femoral artery (distal). The distance and transit time between these two points was determined in relation to the R wave of the electrocardiogram (in m/s).

2.4. PET/CT imaging

Total body Na [^{18}F]F PET/CT scans were performed on a Biograph Vision scanner (Siemens Healthineers, Erlangen, Germany). Patients with T2DM were administered an intravenous injection of 2.0 MBq/kg Na [^{18}F]F 90 min before imaging (defined dose for the current study) and G1-2A1 non-diabetic controls 3.0 MBq/kg Na [^{18}F]F 60 min before imaging (clinical protocol). We accepted these differences between the clinical protocol and study protocol since a previously performed study showed that Na [^{18}F]F activity in the arterial wall and blood pool was similar at 45 and 90 min acquisition and did not improve when the PET scans were performed 180 min after contrast injection [25]. A continuous breathing low-dose CT (80–120 kV, 20–35 mAs, and adjacent 5 mm slices) was performed for visualization of anatomical structures and

calcification assessment, and used as an attenuation correction map. PET acquisitions were obtained with 2–3 min per bed position in 3D setting. Images were reconstructed according to the European Association of Nuclear Medicine guidelines [26], using EARL settings and a time-of-flight iterative reconstruction method (3 iterations, 21 subsets, and voxel size of 3.18 × 3.18 × 2 mm) with point spread function correction. Images were corrected for random coincidences, scatter, and attenuation and were smoothed with a Gaussian filter with 6.5 mm full width at half maximum. Images were reformatted into axial, coronal, and sagittal views, and reviewed using the software provided by the manufacturer (Syngo.Via, Siemens Healthineers, Erlangen, Germany).

2.5. Image analyses

The PET/CT scans were analyzed blinded to patient characteristics and clinical information by decoding the scans with an anonymous study number and scan date.

2.6. Na [^{18}F]F analysis

Two scores were used in the Na [^{18}F]F PET analysis: the grading score and the target-to-background ratio (TBR). First, the femoral artery and aortic grading scores were obtained following the vasculitis grading score [27]. In short, for the femoral arteries a standardized 0–3 grading system was used as follows: 0 = no activity (≤adjacent muscle tissue), 1 = low-grade activity (<femur), 2 = intermediate-grade activity (=femur), 3 = high-grade activity (>femur), as shown in Fig. 1. This grade was multiplied by the length of the arterial positivity (in % of the total upper leg length). The scores of the left and right femoral arteries were summed up to a 'femoral artery grading score'. Since Na [^{18}F]F activity in the spine is substantially higher than aortic uptake, the spine is, in contrast to the femur, not valid to compare aortic activity with. Therefore the 'aortic grading score' was scored as the total length of the arterial positivity (% from aortic arch until the aortic bifurcation).

Besides the femoral artery and aortic grading scoring, Na [^{18}F]F activity in the abdominal aorta and iliac artery was assigned by drawing volumes of interest around the walls of the abdominal aorta (from most caudal renal artery branch until the aortic bifurcation) and both iliac trajectories (common iliac artery and external iliac artery until ileofemoral ligament). Within these volumes, mean standardized uptake value (SUV_{mean}) was calculated using Affinity 2.0 (Hermes Medical Solutions). SUV_{mean} values were corrected for background by dividing the SUV_{mean} by the averaged SUV_{mean} value of two volumes of interest in the mid-lumen blood pool of the inferior caval vein and expressed as target-to-background ratio (TBR). For the iliac trajectories a $_{mean}$TBR was calculated by averaging TBR of the left and right iliac trajectory. Femoral artery grading score and TBR measurements of the abdominal aorta and iliac trajectories were analyzed twice, by two trained observers (MR and JKES). No significant differences in PET imaging results were found between observers, expressed as intraclass correlation coefficient (ICC); the interobserver reproducibility of the femoral artery grading score was 0.38. ICC of Na [^{18}F]F abdominal aorta TBR was 0.70 and ICC of and Na [^{18}F]F iliac trajectories $_{mean}$TBR was 0.02.

2.7. CT-analysis

CT-assessed arterial macro-calcifications were analyzed using two methods: a semi-quantitative score and quantitative measurements. The semi-quantitative score was rated based on the most calcified slice in ten arteries in four individual segments: carotid arteries (2x) (segment 1), ascending aorta and aortic arch (segment 2), descending and abdominal aorta (segment 3), and iliac (2x) and femoral arteries (2x) (segment 4), using the Rominger method [28]. With this method, a calcified plaque (CP) score between 0 and 4 was assigned for the burden of arterial wall calcification (0: no plaque, 1: CP <10%, 2: CP 10–25%, 3: CP 25–50%, 4: CP >50%). To calculate a total CP score for the arterial tree, the scores of all 10 arteries were summed up and expressed as $_{total}$Cpscore with a maximum of 40.

The amount of CT-assessed macro-calcifications was quantified as mass (in mg), volume (in mm^3) and Agatston score using Aquarius iNtuition viewer Version 4.4.13.P6 (TeraRecon, Durham, NC, USA). The thresholds for arterial macro-calcifications were set at 147, 130, and 120 Hounsfield units (HU) for tube voltages of 100, 120 and 140 kVp, respectively, according to literature and the mass calibration was set at 0.84 [29,30]. Macro-calcification plaques were selected in the earlier mentioned ten arteries and quantified. CT-assessed macro-calcification data were expressed as 'CT mass/volume/Agatston score in the femoral arteries' and 'CT mass/volume/Agatston score in 10 arteries of the total aortic tree' (including the earlier mentioned ten arteries of four segments).

2.8. Statistical analyses

Discrete variables are presented as numbers with a percentage of total. Quantitative variables with a non-skewed distribution are presented as mean ± standard deviation (SD) and skewed distributed as median with interquartile range [IQR]. One-way ANOVA with post-hoc t-tests or Kruskal-Wallis with post-hoc Mann-Whitney U tests were performed to test differences between groups. Interobserver PET measurements were assessed with One Way Random absolute agreement single measures ICC Reliability Analysis. Post-hoc Cohen's effect size was calculated between groups based on mean and SD. All statistical analyses were performed with IBM Statistical Package for Social Sciences (SPSS) version 23. $p < 0.05$ was considered as significant.

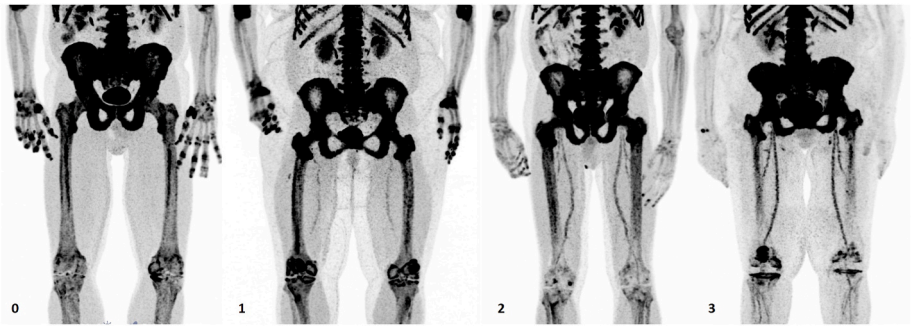

Fig. 1. Femoral artery grading score (0–3) of sodium [^{18}F]fluoride (Na [^{18}F]F) activity in categories.
For the grading scoring a standardized 0-to-3 grading system was used as following: 0 = no activity (≤adjacent muscle tissue), 1 = low-grade activity (<femur), 2 = intermediate-grade activity (=femur), 3 = high-grade activity (>femur) [27].

3. Results

3.1. Patient characteristics

In total, 40 participants were included in this study, consisting of 30 patients with T2DM and 10 G1-2A1 non-diabetic controls. Triglycerides and triglyceride/HDL ratio were significantly higher in G4A3 with T2DM compared with G1-2A1 with T2DM (2.87 [1.78–5.25] mmol/L *vs.* 1.49 [1.35–1.69] mmol/L ($p = 0.025$) and 2.9 [1.6–7.7] *vs.* 1.4 [1.0–1.7] ($p = 0.028$), respectively). G1-2A1 with T2DM showed a significantly lower $HbA1_{1C}$ compared with G1-2A3 with T2DM (52 [44–56] *vs.* 73 [54–82] mmol/mol ($p = 0.019$), but no other differences in patient characteristics were found between groups, as demonstrated in Table 1.

3.2. Albuminuria and arterial disease

3.2.1. Aortic stiffness

PWV was 1.3 times higher in G1-2A3 with T2DM and 1.4 times higher in G4A3 with T2DM compared with G1-2A1 with T2DM (respectively 11.15 [9.45–14.34] ($p = 0.036$) and 12.35 [11.19–14.53] ($p = 0.001$) *vs.* 8.86 [7.79–9.70] m/s, as shown in Table 2 and Fig. 2A). A trend ($p = 0.084$ in Kruskal-Wallis test) of higher pulse pressures was found in both A3 groups with T2DM compared with G1-2A1 with T2DM (67 [57–71] and 72 [58–86] *vs.* 57 [51–65] mmHg, as shown in Table 2).

3.2.2. Arterial Na [^{18}F]F-assessed micro-calcification

The femoral artery grading score was significantly increased in both A3 groups with T2DM (228 [100–446] G1-2A3 with T2DM, and 198 [113–578] in G4A3 with T2DM *vs.* 75 [0–200] in G1-2A1 non-diabetic controls, and 33 [0–93] in G1-2A1 with T2DM ($p = 0.001$)), as shown in Fig. 2B and visualized in Fig. 3. Additionally, the between-group tests of the femoral artery grading score showed a significant difference between G1-2A1 with T2DM and G1-2A3 with T2DM ($p = 0.002$), with a 6.9 times higher score for G1-2A3 with T2DM. The femoral artery grading score showed a trend towards significance in favor of A3 with T2DM compared with G1-2A1 non-diabetic controls ($p = 0.052$). No difference was found between G1-2A1 with T2DM and G1-2A1 non-diabetic controls ($p = 0.218$).

Aortic grading score was significantly higher in G4A3 patients with T2DM compared with those with G1-2A1 with T2DM and G1-2A3 with T2DM (47.5 [33.8–73.8] *vs.* 17.5 [8.8–27.5] ($p = 0.006$) and 25 [10–36] ($p = 0.034$), respectively). Na [^{18}F]F TBR of the abdominal aorta and $_{mean}$TBR of iliac trajectory did not differ between groups.

3.3. Arterial CT-assessed macro-calcification

CT-assessed macro-calcification mass, volume, and Agatston scores in the femoral arteries were increased in G4A3 patients with T2DM compared with G1-2A1 patients with T2DM, as shown in Table 2. In contrast, semi-quantitative CT-assessed macro-calcifications in femoral arteries and total aortic tree did not differ between the four groups.

4. Discussion

In the last decade Na [^{18}F]F was demonstrated to be of additional value in the clinical assessment of atherosclerotic microcalcification. Here we investigated whether albuminuria coincides with harbingers of arterial disease using PWV and micro- and macrocalcification Na [^{18}F]F PET/CT imaging. This study demonstrates that microcalcification markers were significantly increased in patients with T2DM with preserved kidney function having severe albuminuria (G1-2A3) when compared to patients with T2DM with preserved kidney function without albuminuria (G1-2A1). In patients with T2DM patients with severely decreased GFR (G4A3), Na [^{18}F]F activity in the aorta and macro-calcification in the femoral arteries is increased. These data support our hypothesis that albuminuria is a predictor of the presence of subclinical arterial disease.

Albuminuria is a well-known risk factor in relation to early onset CVD, although the pathophysiological mechanisms are not fully understood [31]. We do not observe increased aortic Na [^{18}F]F activity in G1-2A3 patients with T2DM which might be explained by the difference in composition of the arterial wall, when comparing the aorta with the femoral arteries. The aorta is an elastic artery, containing a relatively high amount of elastin and less smooth muscle cells, in comparison with the femoral arteries, which contain mainly smooth muscle cells [32]. We therefore suggest that albuminuria is a proxy for early muscular (i.e.,

Table 1
Clinical characteristics of the study populations.

Total N = 40, Per group N = 10	G1-2A1 non-diabetic controls	G1-2A1 with T2DM	G1-2A3 with T2DM	G4A3 with T2DM	*p*-value
Age (years)	71 [64–73]	69 [64–74]	61 [52–67]	72 [67–76]	
Men (%)	7 (70)	7 (70)	5 (50)	6 (60)	
Diabetes duration (years)		5.0 [4.8–11]	19 [12–24]	23 [13–30]	$\mathbf{p = 0.009}^{a,b}$
Insulin use (%)		0	100	100	$\mathbf{p < 0.001}^{a,b}$
Statin use (%)		7 (70)	6 (60)	6 (60)	$p = 0.870$
Anti-hypertensive drug use (%)		7 (70)	10 (100)	9 (90)	$p = 0.114$
Estimated Glomerular Filtration Rate (mL/min/1.73m^2)	91 [81–104]*	87 [84–93]	85 [60–103]	19 [13–27]	
Albumin-Creatinine Ratio (mg/mmol)		0.35 [0.25–0.75]	74 [62–122]	131 [59–304]	
Body Mass Index (kg/m^3)		31.3 ± 3.7	31.0 ± 5.3	35.4 ± 4.5	$p = 0.089$
Smoking (%)		2(20)	2(20)	1(10)	$p = 0.212$
Calcium (mmol/L)		2.38 ± 0.05	2.37 ± 0.11	2.31 ± 0.10	$p = 0.279$
Phosphate (mmol/L)		0.95 ± 0.21	0.92 ± 0.16	1.25 ± 0.41	$p = 0.100$
Cholesterol (mmol/L)		4.15 [3.68–5.10]	5.10 [3.38–5.85]	4.40 [3.55–5.53]	$p = 0.814$
HDL cholesterol (mmol/L)		1.10 [0.98–1.33]	0.95 [0.80–1.23]	0.90 [0.68–1.13]	$p = 0.135$
LDL cholesterol (mmol/L)		2.99 ± 1.02	2.71 ± 1.15	2.35 ± 0.84	$p = 0.497$
Triglycerides (mmol/L)		1.49 [1.35–1.69]	1.94 [1.03–4.38]	2.87 [1.78–5.25]	$\mathbf{p = 0.029}^{a}$
HbA_{1C} (mmol/mol)		52 [44–56]	73 [54–82]	65 [48–75]	$\mathbf{p = 0.032}^{b}$
HOMA-insulin resistance		5.13 [3.68–12.80]	12.45 [7.78–23.08]°	13.69 [7.89–29.10]	$p = 0.058$
Triglycerides/HDL ratio		1.4 [1.0–1.7]	2.3 [0.9–5.1]	2.9 [1.6–7.7]	$\mathbf{p = 0.032}^{a}$

Clinical characteristics of four sub groups: G1-2A1 non-diabetic controls (controls without diabetes or albuminuria), G1-2A1 patients with type 2 diabetes mellitus (T2DM), G1-2A3 patients with T2DM and severely increased albuminuria, and G4A3 patients with T2DM, severely increased albuminuria and severely decreased kidney function. Values are N (percentage of the group), mean ± standard deviation or median [and interquartile range]. *N = 8, °N = 9. HDL = High-Density Lipoprotein, LDL = Low-Density Lipoprotein. Additional Mann-Whitney U tests with $p < 0.05$.

[a] = G4A3 with T2DM *vs.* G1-2A1 with T2DM.

[b] = G1-2A3 with T2DM *vs.* G1-2A1 with T2DM.

Table 2
Markers of arterial disease in the study populations.

Total N = 40, Per group N = 10	G1-2A1 non-diabetic controls	G1-2A1 with T2DM	G1-2A3 with T2DM	G4A3 with T2DM	*p*-value
Pulse pressure (mmHg)		57 [51–65]	67 [57–71]	72 [58–86]	$p = 0.084$
PWV (m/s)		8.65 [7.79–9.70]	11.15 [9.45–14.34]	12.35 [11.19–14.53]	$\boldsymbol{p = 0.001}$[a,b]
Femoral artery grading score (Na [^{18}F]F)	75.0 [0.0–200.0]	32.5 [0.0–92.5]	227.5 [100.0–446.3]	197.5 [112.5–577.5]	$\boldsymbol{p = 0.001}$[a,b]
Aortic grading score (Na [^{18}F]F)	27.5 [10.0–50.0]	17.5 [8.8–27.5]	25.0 [10.0–36.3]	47.5 [33.8–73.8]	$\boldsymbol{p = 0.006}$[a,c]
Na [^{18}F]F TBR abdominal aorta	1.27 [1.06–1.82]	1.83 [1.47–1.96]	1.52 [1.31–1.70]	1.70 [1.47–2.03]	$p = 0.114$
Na [^{18}F]F $_{mean}$TBR iliac trajectories	1.16 [0.90–1.52]	1.49 [1.35–1.59]	1.30 [1.08–1.57]	1.49 [1.25–1.70]	$p = 0.226$
Semi-quantitative score $_{total}$CP	15.0 [7.0–22.8]	20.0 [7.5–24.5]	16.5 [11.8–22.0]	22.5 [14.5–27.8]	$p = 0.355$
Semi-quantitative score CP iliac and femoral arteries	7.5 [2.8–13.0]	9.0 [3.0–12.0]	9.0 [6.5–11.0]	10.5 [7.8–13.0]	$p = 0.452$
CT mass total aortic tree (mg)	2036 [319–4477]	711 [88–2188]	756 [96–2586]	2588 [742–3586]	$p = 0.317$
CT volume total aortic tree (mm^3)	7146 [1008–16228]	3260 [500–8799]	2977 [499–11441]	10847 [3600–14811]	$p = 0.280$
CT Agatston score total aortic tree	8895 [1199–18953]	3625 [486–10573]	3447 [339–13328]	13093 [2965–16653]	$p = 0.289$
CT mass femoral arteries (mg)	37.0 [0.0–609.3]	12.2 [1.41–44.3]	23.6 [0.0–559.5]	291 [170–511]	$\boldsymbol{p = 0.024}$[a]
CT volume femoral arteries (mm^3)	97.9 [0.0–2452.0]	62.6 [10.8–675.8]	125.8 [0.0–2329.8]	1463 [1151–2776]	$\boldsymbol{p = 0.026}$[a]
CT Agatston score femoral arteries	97.3 [0.0–2590.0]	46.5 [1.4–671.0]	105.6 [0.0–2774.0]	1304 [639–2456]	$\boldsymbol{p = 0.036}$

Measurements of arterial disease in four sub groups: G1-2A1 non-diabetic controls (controls without diabetes or albuminuria), G1-2A1 patients with type 2 diabetes mellitus (T2DM), G1-2A3 patients with T2DM and severely increased albuminuria, and G4A3 patients with T2DM, severely increased albuminuria and severely decreased kidney function. PWV = pulse wave velocity, Na [^{18}F]F = sodium [^{18}F]fluoride, TBR = target to background ratio, CP = calcified plaque, CT = computed tomography.
Additional Mann-Whitney U tests with $p < 0.05$.
[a] = G4A3 with T2DM *vs.* G1-2A1 with T2DM.
[b] = G1-2A3 with T2DM *vs.* G1-2A1 with T2DM.
[c] = G4A3 with T2DM *vs.* G1-2A3 with T2DM.

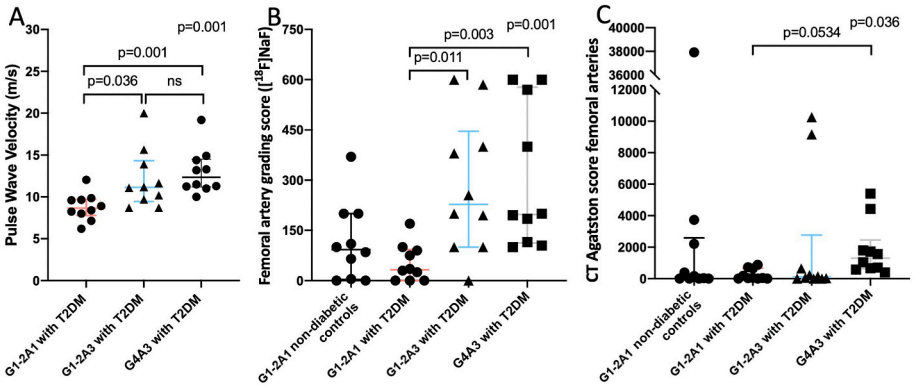

Fig. 2. Patients with type 2 diabetes mellitus with severely increased albuminuria show increased pulse wave velocity and increased sodium [^{18}F]fluoride (Na [^{18}F]F) activity in femoral arteries.
Carotid-femoral pulse wave velocity-assessed aortic stiffness was higher in patients with type 2 diabetes mellitus (T2DM) with severely increased albuminuria (albumin-creatinin ratio (ACR) > 30 mg/mmol, defined as KDIGO A3), and in A3 patients with T2DM severely decreased kidney function (estimated glomerular filtration rate (eGFR) mL/min/1.73 m^2 between 15 and 30, defined as KDIGO G4) compared with G1-2A1 patients with T2DM (A). Femoral artery grading was higher in G1-2A3 with T2DM and in G4A3 with T2DM compared with G1-2A1 patients with T2DM (B). G1-2A3 patients with T2DM showed an almost significant trend of increased computed tomography (CT)-assessed Agatston score in the femoral arteries compared with G1-2A1 patients with T2DM (C). Kruskal-Wallis tests were used to compare the multiple groups. If the Kruskal-Wallis test showed a significant difference, additional Mann-Whitney U tests were performed to test differences between groups. In (B), only p values below 0.05 were shown.

tunica media) micro-calcification in the tunica media of the arterial wall. Except for a single report from 2015 [33], increased activity of Na [^{18}F]F in the femoral arteries in patients with severely decreased kidney function or T2DM was not demonstrated before. Our results have clinical implications since media sclerosis is irreversible in an advanced stage, which underlines the importance of targeting albuminuria to prevent the progression from micro-calcification to macro-calcification with its corresponding increased morbidity and mortality.

Although clinical studies with Na [^{18}F]F in patients with T2DM are limited, our results are in line with other studies. Na [^{18}F]F activity in femoral arteries was found to be associated with several modifiable cardiovascular risk factors such as total cholesterol, triglycerides, HbA_{1C} in patients with T2DM and calcified plaque burden, and peripheral arterial disease in patients at high risk for CVD [34–36]. Furthermore, it has been reported that Na [^{18}F]F activity in femoral arteries was associated with lower limb restenosis after percutaneous transluminal angioplasty [36]. Recently it was demonstrated that coronary artery Na [^{18}F]F uptake predicts the progression of calcifications in patients with diabetes [37].

Albuminuria was defined both as an indicator and a contributor to impaired endothelial function, and albuminuria reduction was associated with fewer cardiovascular and kidney disease outcomes, independent of other treatment effects [31,38]. Furthermore, in the last decade it was demonstrated that low-sodium diet, renin angiotensin aldosterone system (RAAS) inhibitors and sodium-glucose cotransporter 2 (SGLT2) inhibitors decrease albuminuria and progression of CKD and possibly confer independent prevention of CVD [31,39,40]. Our results are in line with these intervention studies stressing the importance of targeting albuminuria in high-risk patients with T2DM and demonstrating the tight relation between albuminuria and the vascular pathology early in the process.

The PWV-assessed aortic stiffness is increased in the A3 groups, but not in the G1-2A1 patients with T2DM. This suggests that albuminuria also affects the aortic wall. It has also been shown that PWV is associated with peripheral arterial stenosis severity, indicating that it is a marker of systemic arterial disease [41]. While PWV provides information about systemic arterial disease, local atherosclerosis (e.g. IMT) can be measured with B-mode ultrasound [13]. IMT, PWV and pulse pressure measurements, however, do not provide information about the atherogenic molecular processes in the arterial wall. This suggests that aortic stiffness is increased in an early phase of the development of arterial calcification, when micro-calcification deposits are present, stiffening

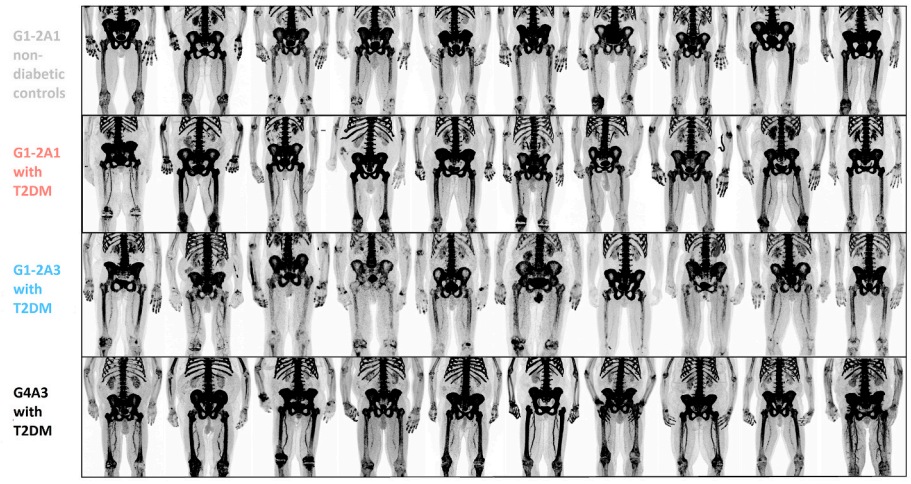

Fig. 3. Patients with type 2 diabetes mellitus with severely increased albuminuria show increased sodium [^{18}F] fluoride (Na [^{18}F]F) activity in femoral arteries. Femoral artery grading scoring of Na [^{18}F]F activity of the four different groups. First row: ten G1-2A1 non-diabetic controls, second row: ten G1-2A1 patients with type 2 diabetes mellitus (T2DM), third row: ten G1-2A3 patients with T2DM, fourth row: ten G4A3 patients with T2DM.

the arteries which then results in hypertension.

In the current study we showed that G4A3 patients with T2DM showed increased CT-assessed femoral macro-calcifications. This is not found in G1-2A3 patients with T2DM. It is well-known that a decreased kidney function increases the risk for developing CVD [6]. However, the fact that the presence of albuminuria in patients with T2DM is associated with subclinical arterial disease (i.e., micro-calcification and aortic stiffness), but not advanced arterial disease (i.e. macro-calcifications), has not been shown before. The current study therefore supports the hypothesis that the presence of albuminuria is a proxy for subclinical, potentially reversible, atherosclerosis in patients with T2DM.

This study has a few limitations that need to be addressed. Firstly, SUV_{mean} measurements in femoral arteries were not possible because the LDCT anatomy had insufficient quality to detect the femoral arteries on PET/CT overlay images. Therefore, we used the grading scoring method to get a reliable value of Na [^{18}F]F activity in femoral arteries. Secondly, no correction for statin use, or other drugs could be added because of the small sample size, even though statins may affect the correlations between cholesterol and arterial measurements. Finally, since this study was designed as a descriptive pilot study, no sample size calculations were performed in advance. A post-hoc Cohen's effect size was calculated 1.5 between G1-2A1 patients compared with G1-2A3 patients and was 1.6 between G1-2A1 patients and G4A3 patients.

In conclusion, this study demonstrates that subclinical arterial disease, in terms of micro-calcification and aortic stiffness, is increased in G1-2A3 patients with T2DM. In those with additional, severely decreased, kidney function, also macro-calcifications are found. Since the development of CVD can potentially be inhibited early in the trajectory of the disease, albuminuria might be an important target. Medical drug interventions such as SGLT2 inhibitors, should be considered to be implemented in the treatment of patients with T2DM with albuminuria. Since arterial damage develops in an early phase of the disease, these interventions should be implemented before severe decreased kidney function occurs.

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Author contributions

M.R. collected, analysed, interpreted data, and wrote the manuscript, J.K.E.S. collected, analysed, interpreted data, conceived and designed the study and reviewed the manuscript critically for intellectual content, C.A.V.K., M.H.B., P.R.D., and K.H. defined the study participant groups, included participants and reviewed the manuscript critically for intellectual content, G.D.P., M.J.W.G., and G.L. designed the technical methods and analysed, interpreted data, conceived and designed the study and reviewed the manuscript critically for intellectual content. H.H.B., R.A.P., and J.L.H. conceived and designed the study and reviewed the manuscript critically for intellectual content, D. J.M. collected, analysed, interpreted data, conceived and designed the study and reviewed the manuscript critically for intellectual content. M. R. and R.H.J.A.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the 2 integrity of the data and the accuracy of the data analysis. All authors approved the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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