

Original Article

An understanding of the atherosclerotic molecular calcific heterogeneity between coronary, upper limb, abdominal, and lower extremity arteries as assessed by NaF PET/CT

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Abstract: We aimed to quantify the heterogeneity of atherosclerosis in upper and lower limb vessels using ¹⁸F-NaF-PET/CT and compare calcification in coronary arteries to peripheral arteries. 68 healthy controls (42±13.5 years, 35 females, 33 males) and 40 patients at-risk for cardiovascular disease (55±11.9 years, 22 females, 18 males) underwent PET/CT imaging 90 minutes after the injection of ¹⁸F-NaF (2.2 Mbq/Kg). The following arteries were examined: coronary artery (CA), ascending aorta (AS), arch of aorta (AR), descending aorta (DA), abdominal aorta (AA), common iliac artery (CIA), external iliac artery (EIA), femoral artery (FA), popliteal artery (PA). Average SUVmean (aSUVmean) was calculated for each arterial segment. A paired t-test compared the aSUVmean between CA vs. AS, AR, DA, AA, CIA, EIA, FA, and PA. CA aSUVmean in the at-risk group was higher than the healthy control group (0.74±0.04 vs. 0.67±0.04, P=0.03). Furthermore, the ¹⁸F-NaF uptake in the CA was lower than in AS, AR, DA, AA, CIA, EIA, FA, and PA in both healthy (all P≤0.0001) and at-risk (all P≤0.0001). Higher ¹⁸F-NaF uptake in non-cardiac arteries in both healthy controls and patients at-risk suggests CA calcification is a late manifestation of atherosclerosis. This differential expression of atherosclerosis is likely due to interaction of hemodynamic parameters specific to the vascular bed and systemic factors related to the development of atherosclerosis.

Keywords: Atherosclerosis, ¹⁸F-NaF, positron emission tomography, computed tomography

Introduction

Atherosclerosis is a chronic pathological disease characterized by a buildup of lipid-based plaque from inflammation and subsequent calcification of the arterial lining [1]. Correlations between calcification severity and cardiovascular disease (CVD) have been extensively studied in various vessels throughout the body [2], but there lacks adequate information regarding the heterogeneity of calcification amongst the different vascular beds. Understanding this heterogeneity is crucial for monitoring CVD progression and address the condition early.

Calcification can be effectively visualized using a number of imaging modalities. Computer Tomography (CT) is the current clinically accepted standard of care for structural detection due to its ability to accurately depict calcification that precedes plaque creation [3], though other structural imaging modalities like magnetic resonance imaging and ultrasonography have also proven effective in visualizing affected vasculature [4, 5]. However, these methodologies lack precision for microcalcifications that appear at the early stages of atherosclerosis; furthermore, such methodologies are unable to assess the progression of existing calcification. Thus,

positron emission tomography/computed tomography (PET/CT) may serve as the superior imaging technique as it contains both precise and accurate detection of atherosclerosis at early stages and throughout disease progression [6].

Recent evidence has suggested that ^{18}F -sodium fluoride (^{18}F -NaF)-PET is among the best radiopharmaceuticals at detecting intravascular calcification as opposed to more commonly used ^{18}F -fluorodeoxyglucose (FDG)-PET [7-9]. Current studies have proven ^{18}F -NaF-PET/CT in coronary artery calcification [10], but only a few studies have investigated ^{18}F -NaF-PET in the peripheral arteries. Thus, the aim of this study is two-fold: 1. Use ^{18}F -NaF-PET/CT to quantify the heterogeneity of atherosclerosis in various vascular beds of upper and lower limb extremities, and 2. Assess the association between coronary arteries and vessels of upper and lower limb vessels hypothesizing that the atherosclerotic burden is higher in non-coronary vasculature and moreover, that the burden is higher in patients at-risk for CVD than healthy subjects.

Methods

Patient population

Subjects from the Cardiovascular Molecular Calcification Assessed by ^{18}F -NaF-PET/CT (CAMONA) trial (ClinicalTrials.gov (NCT01724749)) were recruited as both atherosclerosis and healthy control patients in this study. The healthy controls were identified as patients who did not have any existing disease, cardiovascular risk factors, or drug-abuse issues. The Systemic Coronary Risk Evaluation (SCORE) tool was used to exclude any patient who had a 10-year risk for fatal cardiovascular disease below 1%. All trial participants provided written consent. This study identified 40 patients at risk for cardiovascular disease (55 ± 11.9 years, 22 females, 18 males) and 68 healthy controls (42 ± 13.5 years, 35 females, 33 males) that contained full-body arterial imaging without artifact of CAMONA patients for analysis.

Image acquisition

Clinically accepted standardized protocols were followed for image acquisition. Full body ^{18}F -NaF PET/CT images were acquired using inte-

grated PET/CT scanners (General Electric Discovery RX, STE, 690/710 imaging, Chicago, Illinois). Both healthy control and atherosclerosis patients were injected with 2.18 MBq/kg of ^{18}F -NaF and subsequently received at PET/CT scans after 90 minutes. Low-dose CT scans were preferred to reduce radiation applied while leveraging attenuation correction for anatomical localization of PET images.

Image analysis

Osirix MD (version 7.04; Pixmeo SARL, Bernex, Switzerland) was used for image analysis. PET images were fused and overlaid on CT images to provide anatomical localization and a better understanding of ^{18}F -NaF uptake in plaque. Regions of interest (ROI) were drawn by an experienced physician to determine standard uptake values (SUV) of each slice of the following arteries: coronary (CA), ascending aorta, arch of aorta, descending aorta, abdominal aorta, common iliac, external iliac, femoral, and popliteal. CA ROIs were placed around the cardiac structure while excluding aortic valve, cardiac valves, and nearby skeletal structures (**Figure 1**). Abdominal and peripheral arteries were analyzed using previously established ^{18}F -NaF-PET/CT methodology (**Figure 2**) [11-13]. Average SUVmean (aSUVmean) was calculated for each arterial segment in controls and patients.

Statistics

Descriptive statistics were derived according to data type: continuous variables were displayed as mean and standard deviation, categorical variables as frequencies and respective percentages. Box-and-whisker plots were employed for visualization purposes. Paired t-tests comparing the aSUVmean between coronary and all peripheral arteries within patient groups were done; intergroup comparisons were performed with unpaired t-tests. Statistical analysis was done in R [14].

Results

Patient demographics were identified for both healthy and at-risk patients (**Table 1**). Our results have shown that aSUVmean of CA was higher in the at-risk group as compared to healthy controls (0.74 ± 0.12 vs. 0.68 ± 0.16 , $P=0.03$). Furthermore, the ^{18}F -NaF uptake in

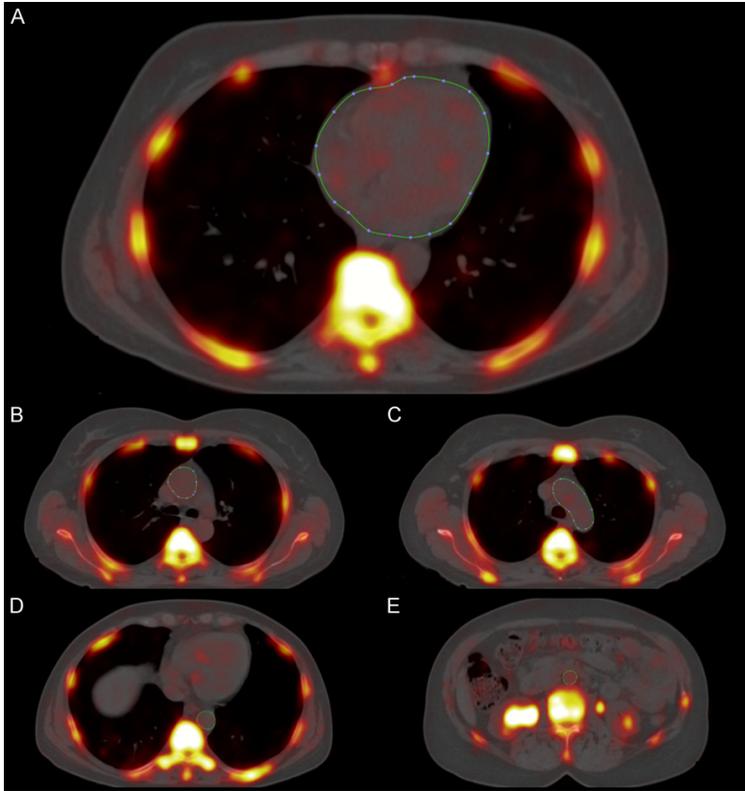


Figure 1. Axial fused NaF-PET/CT with regions of interest (ROI) showing upper limb arteries in a 47-year-old female at-risk for CVD. Drawn ROI's determined NaF uptake in (A) global coronary arteries (which excluded aortic valve, skeletal structures, and aortic wall), (B) ascending aorta, (C) aortic arch, (D) descending aorta, and (E) abdominal aorta.

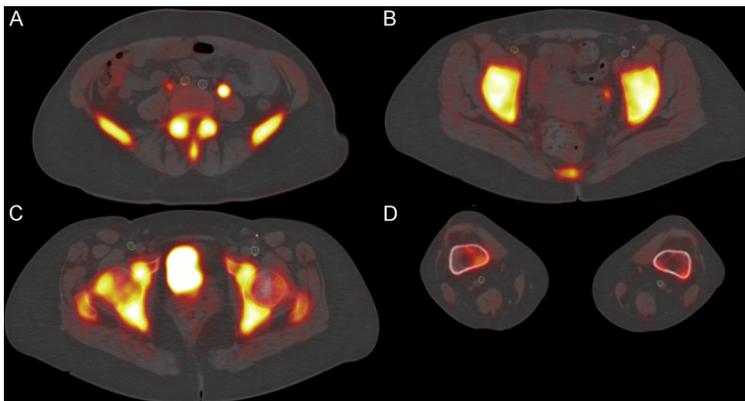


Figure 2. Axial fused NaF-PET/CT with regions of interest (ROI) showing lower limb arteries in a 47-year-old female at-risk for CVD. Drawn ROI's determined NaF uptake in (A) common iliac artery, (B) external iliac artery, (C) femoral artery, and (D) popliteal artery.

the abdominal and lower limb vessels was found to be higher than that found in the CA for both healthy and at-risk patients (all $P < 0.01$, **Table 2**). We found that ^{18}F -NaF uptake was

higher in the abdominal and lower limb vessels as compared to the heart in both healthy controls and patients at risk for CVD (**Figure 3**).

Discussion

In this study, we hypothesized that vascular beds downstream from the heart would contain a greater atherosclerotic burden than the coronary arteries. These results suggest calcification in the coronary arteries has later manifestation than that of the peripheral arteries in the atherosclerotic disease progression. This difference can perhaps be attributed to the change in hemodynamic pressures.

There is no surprise that the atherosclerotic burden was higher in vessels with greater pressure. Arterial pressure and calcification are cyclically related: as a patient's vascular pressure increases, there is an increased inflammatory response causing focal stenosis [15]. The arteries measured in this study follow the same general stream from the ascending aorta to popliteal arteries. The aorta's proximity to the left ventricle allows it to use ventricular force to pump the blood throughout the body while the lower limb vessels are often more muscular and induce local pumping for the distribution of blood to the capillaries [16]. By contrast, coronary perfusion pressure relies on the pressure gradient between the aorta and left ventricle at end-diastole [17]. This intrinsic anatomical difference implies there is a greater chance for atherosclerotic burden

in the peripheral arteries from greater induced pressures and can explain the gradient of increased calcification as blood flows from ascending aorta to the common iliac (**Table 1**).

Atherosclerotic heterogeneity by NaF

Table 1. Subject demographics

	Healthy Total (N=68)	High-Risk Total (N=40)
Age	41.7±13.5	55.4±11.9
Systolic blood pressure (mm Hg)	126.7±14.5	130.0±17.5
Diastolic blood pressure (mm Hg)	75.5±9.4	78.8±7.5
Pulse (beats/minute)	65.2±11.2	62.2±12.2
Low density lipoprotein (mmol/L)	3.0±0.8	3.4±0.8
Total cholesterol (mmol/L)	4.8±0.8	5.3±0.9
Triglycerides (mmol/L)	1.0±0.6	1.2±0.8
HDL cholesterol (mmol/L)	1.4±0.4	1.4±0.5
Plasma glucose (mmol/L)	5.4±0.4	5.6±0.7
HbA1c (mmol/L)	33.1±3.7	34.7±3.1
CRP (mg/L)	2.2±3.3	2.5±3.2

Note: Values are mean ± SD. HbA1c = Glycated hemoglobin.

Table 2. Comparing NaF uptake between coronary and peripheral arteries in healthy and at-risk patients

Vascular segment	Uptake (aSUVmean)	Difference from Coronary Artery (aSUVmean)	P-values
Healthy controls			
Coronary Artery	0.67±0.04		
Ascending Aorta	0.81±0.05	0.13±0.06	P<0.0001
Arch of Aorta	0.82±0.06	0.14±0.07	P<0.0001
Descending Aorta	0.92±0.10	0.24±0.10	P<0.0001
Abdominal Aorta	1.32±0.09	0.64±0.10	P<0.0001
Common Iliac	2.41±0.14	1.72±0.15	P<0.0001
External Iliac	1.78±0.10	1.10±0.11	P<0.0001
Femoral	1.47±0.08	0.79±0.09	P<0.0001
Popliteal	1.38±0.08	0.70±0.09	P<0.0001
Patients at risk			
Coronary Artery	0.74±0.04		
Ascending Aorta	0.98±0.09	0.30±0.10	P<0.0001
Arch of Aorta	1.02±0.11	0.34±0.12	P<0.0001
Descending Aorta	1.04±0.09	0.35±0.10	P<0.0001
Abdominal Aorta	1.32±0.13	0.64±0.14	P<0.0001
Common Iliac	2.43±0.26	1.74±0.36	P<0.0001
External Iliac	1.79±0.16	1.10±0.16	P<0.0001
Femoral	1.73±0.14	1.04±0.15	P<0.0001
Popliteal	1.56±0.11	0.85±0.12	P<0.0001

Note: Values are mean ± 95% confidence interval.

Comparing the healthy controls to patients at risk of CVD, we see that atherosclerotic burden is generally higher as CVD severity increases due to various risk factors (Table 2). Older patients are at greater risk for arterial calcification as the natural wear and tear of the arteries

deteriorate the lumen and intimal thickening [18]. Further, though high-density lipoprotein amounts aren't different, an increase in low-density lipoproteins is directly associated with increased molecular calcification. Patients at greater risk for CVD often have increasingly western diets, which can contain high cholesterol, and cause fatty streaks in the arterial lumen [19]. These risk factors are further exacerbated by preexisting diabetic symptoms; patients at risk have higher HbA1C scores than healthy patients though this association was not noted in our study. These risk factors all promote the cyclical development of atherosclerotic severity and explain greater ¹⁸F-NaF uptake in at-risk patients. Despite this difference, the trend of greater uptake in peripheral compared to coronary arteries was present in both groups. This supports the hypothesis that increased coronary calcification occurs as atherosclerosis of abdominal and lower limb vessels increases, a previously unexplored association with ¹⁸F-NaF. Such an association is crucial for our understanding of CVD as a whole because the detection of any calcification in peripheral arteries could provide early diagnosis for the CVD.

Despite these findings, there are limitations to note. First, the theory of hemodynamics influencing the degree of calcification cannot properly be verified due to a lack of systemic pressure readings in the CAMONA trial. A Doppler ultrasonic blood flow test can provide estimates of systemic pressure [20]; this information, along with histological confirmation, can help in truly understanding the results observed in this study. Secondly, our study included 108 subjects of which only 40 were at-risk patients, showing a small sample size despite the highly significant results. Lastly, the cross-sectional study design lacks the ability to track disease progression. A future longitudinal follow-up of both controls and at-risk patients may give greater insight into the true difference of calcification

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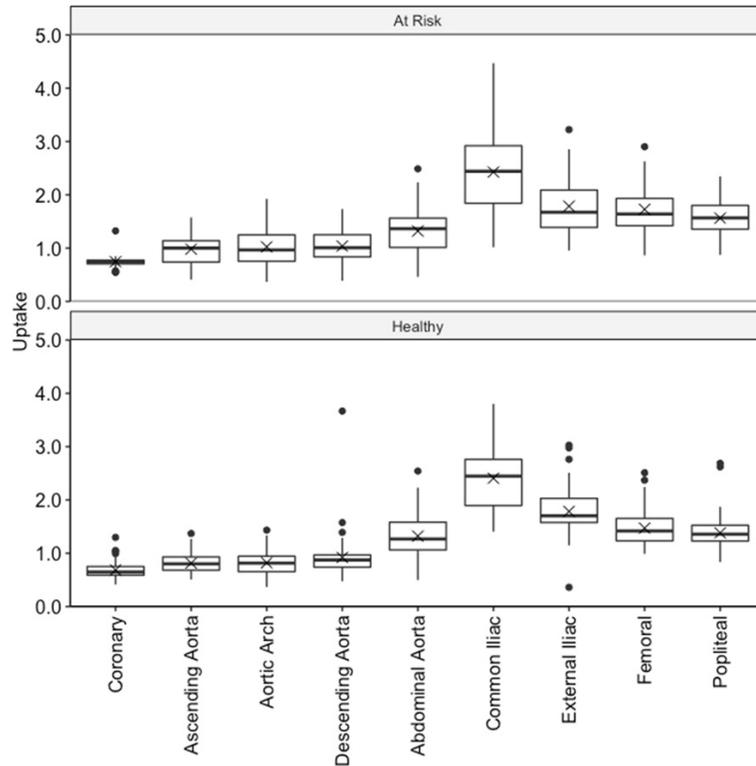


Figure 3. Box plot comparison of uptake as measured in coronary and peripheral arteries. X depicts the mean of data; whiskers depict differences from 1st and 3rd quartile to minimum and maximum respectively; extraneous dots depict outliers. Peripheral arteries had significantly higher uptake than coronary in both at-risk and healthy patients.

in the coronary and peripheral arteries. Nevertheless, this study is among the first to show the important difference in atherosclerotic burden in coronary and peripheral arteries using ^{18}F -NaF. Future studies should further explore this relationship and the potential clinical significance of ^{18}F -NaF-PET/CT imaging for the detection and quantification of atherosclerotic microcalcifications.

In conclusion, our study demonstrated heterogeneous arterial wall microcalcification in major arterial segments of the body and consistently lower ^{18}F -NaF uptake in cardiac compared to non-cardiac arteries in both healthy controls and patients at-risk. In non-cardiac arteries of both groups, there was the same rising and falling pattern of ^{18}F -NaF uptake from the carotid to the popliteal arteries reaching a peak in the iliac arteries.

Disclosure of conflict of interest

None.

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