Association of baseline subject characteristics with changes in coronary calcification assessed by ^{18}F-sodium fluoride PET/CT

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Abstract

Objective: The goal of this study was to test if changes in coronary microcalcification over a two year period assessed by fluorine-18-sodium fluoride (^{18}F-NaF) positron emission tomography/computed tomography (PET/CT) are associated with baseline subject characteristics. **Subjects and Methods:** This prospective study included healthy female (N=8, age 52±10 years, body mass index(BMI) 24±1.7kg/m^2) and male (N=15, age 50±10 years, BMI 27±2.9kg/m^2) participants who had ^{18}F-NaF PET/CT scans taken two years apart. Imaging was performed 90 minutes after intravenous injection of 2.2MBq of ^{18}F-NaF per kilogram of body weight. The analysis regions were selected on CT images by drawing volumes of interest around the entire heart using a semi-automatic segmentation method. Mean standardize uptake value (SUVmean) and maximum SUV (SUVmax) were calculated in the same regions of the registered PET images. Percent change in SUV between the two time points were correlated against baseline age, BMI, cardiovascular risk factors, and blood chemistry. **Results:** High BMI is a known risk factor for atherosclerosis. Our data showed that rate of increase in coronary microcalcification over time measured by ^{18}F-NaF PET/CT is associated with baseline BMI and some clinical risk factors in males. Lack of such associations in females could be due to low sample size (N=8). Further prospective studies are needed to determine if baseline BMI and clinical factors could be used to predict rate of increase in coronary microcalcification which could provide the basis for managing the progression of atherosclerosis in patient-specific manner.

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Introduction

Plaque macrocalcification and microcalcification are associated with atherosclerosis. Atherosclerosis often does not have symptoms, but when severe it can lead to a heart attack or stroke. Plaque microcalcification can lead to plaque vulnerability and increase the potential for the rupture that causes cardiovascular events [1-3].

While computed tomography (CT) imaging is capable of measuring macrocalcification in the coronary arteries it cannot detect microcalcification [1]. Fluorine-18-sodium fluoride (^{18}F-NaF) is a radiopharmaceutical with affinity of fluoride to hydroxyapatite. For this reason, it is appealing for bone imaging. Recent studies have shown that ^{18}F-NaF could also be used to detect calcified micro-deposits within coronary plaque [1, 2, 4-8]. Fluorine-18-NaF positron emission tomography (PET)/CT is the only noninvasive imaging technique currently capable of distinguishing between macro- and microcalcification [2]. Fluorine-18-NaF is able to distinguish between areas of micro and macrocalcification because the extent of fluoride absorption depends on surface area, making it more likely to bind to microcalcification but unable to penetrate deeper into areas of macrocalcification [2]. The method of using ^{18}F-NaF PET/CT scans to detect microcalcification has been shown to be repeatable and reproducible [8].

Fluoride uptake has been shown to correlate with cardiovascular disease risk and have a direct link to myocardial infarction [4, 5, 9]. Many individuals with coronary artery disease are asymptomatic. One study of middle aged Western asymptomatic subjects found that a majority had scans indicating coronary artery disease [10]. Fluorine-18-NaF imaging has been shown to be feasible even in healthy subjects [11]. The disease is often a silent killer, leading to abrupt deaths due to myocardial infarction, but ^{18}F-NaF is associated with such events. In a study of 40 subjects with myocardial infarction, the highest coronary ^{18}F-NaF uptake was seen on culprit plaque [5], revealing the power of the radiotracer to indicate potential risk areas. In addition to being able to identify areas of myocardial infarction, ^{18}F-NaF has been shown to be able to provide an independent predic-

tion of whether myocardial infarction will be fatal or nonfatal [6].

With ^{18}F-NaF being a reliable indicator of microcalcification, progression of atherosclerosis, and the possibility of coronary events, it becomes a powerful tool to study what impacts these occurrences and how to prevent them. Prior studies have examined the factors that uptake of ^{18}F-NaF correlates with, including age, BMI, hypertension, and male sex [7, 12, 13]. Our study examined the rate of change of calcification over a two year period in individuals. Understanding what factors relate to increase in microcalcification can help improve the predictive power of ^{18}F-NaF imaging in healthy subjects. The primary purpose of our study was to test which baseline subject characteristics are associated with the changes in coronary microcalcification over a two year period as assessed by ^{18}F-NaF PET/CT standardize uptake values (SUV).

Subject, Materials, and Methods

The participants of this prospective study were taken form a larger prospective Cardiovascular Molecular Calcification Assessed by ^{18}F-NaF PET/CT (CAMONA) study. The study was conducted by Odense University Hospital and approved by the Danish National Health Committee on Health Research Ethics. Written informed consent was given by all participants, and the study was conducted in accordance with the Declaration of Helsinki.

Participant selection

Our study included healthy subjects from the CAMONA study that came in for PET/CT scans two years apart. The scans were conducted between 2012 and 2015. In order to be considered healthy, subjects needed to have total serum cholesterol below 6.2mmol/L, systolic blood pressure below 160 mm/Hg, diastolic blood pressure below 100mm/Hg, and glycated hemoglobin below 48mmol/mol. Subjects with a diagnosis of type II diabetes or previous history of cardiovascular events were not included. Twenty-three individuals (female, N=8, 52±10 years, BMI 24±1.7 kg/m^2; male, N=15, age 50±10 years, body mass index (BMI) 27±2.9kg/m^2) in the study had two year follow-up data with consistent dosages, and were used to examine the change in SUVmean, SUVmax, and Hounsfield unit (HU) mean over time, as well as the relationship between these changes and age, BMI, cardiovascular risk factors, and blood chemistry. There were initially 29 healthy controls with follow-up CAMONA scans, but one of which do not have a baseline ^{18}F-NaF scan. Of these 28 individuals, five of them had dosages varying by over 10 times the median difference in dose of 1.89% between baseline and follow-up. Since it has been shown that quantification of arterial ^{18}F-NaF is affected by injected dose, these scans were not used to generate estimates of change over time[14].

Study design

Hybrid PET/CT machines were used to create ^{18}F-NaF PET/CT imaging. Subjects were intravenously injected with approximately 2.2MBq of ^{18}F-NaF per kilogram of body weight and images were obtained 90 minutes later. Total body PET images were acquired in 3D-mode and reconstructed into coronal, transverse, and sagittal slices by an iterative reconstruction algorithm (GE VUE Point). Low-dose CT images (140kV, 30-110mA, noise index 25, 0.8s per rotation, slice thickness 3.75mm) were attenuation corrected. All participants had BMI, age, coronary calcium score, cardiovascular risk factors, blood chemistry, and Framingham Heart SCORE determined at the time of their baseline scan. Cardiovascular risk factors included smoking history, alcohol history, blood pressure, heart rate, history of disease, and medication. Blood pressure was measured three times after a rest of at least 30 minutes. Systolic and diastolic blood pressure were determined by the average of the final two measurements. Blood chemistry analyses included a measurement of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, cysteine, fasting plasma glucose, HbA1c, CRP, fibrinogen, white blood cell count, creatinine, and estimated glomerular filtration rate (eGFR). When returning for a follow-up, subjects' BMI were again measured.

To determine calcium scores, regions of calcified plaque were identified by two radiologists. The area of calcium-containing pixels over 130HU were then multiplied by weight (milligram) to quantify visible calcified plaque.

Imaging analysis

The operator guided software PMOD (PMOD Technologies LLC, Switzerland) was used to calculate SUV. Computed tomography and PET images for each patient scan were uploaded to the software and superimposed. The analysis regions were selected by drawing volumes of interest around the heart using a semi-automatic segmentation method. Hand-drawn regions of interest (ROI) were manually delineated around the cardiac silhouette on each 3.75-mm thick coronal slice from the anterior-most aspect to the posterior-most aspect. We insured regions with valvular (aortic and mitral) uptake were omitted. These ROI were combine across the length of the heart to create a 3-dimensional volume of interest (VOI) that encompassed the heart. SUVmean and SUVmax were calculated from each VOI. The same VOI that was used to determine SUV was imposed on the CT to determine HU. This method has been determined valid by 5 researchers who each analyzed 5 of the same images. Inter-operator reproducibility were 1.9% for mean coefficient of variation and 0.997% for intraclass correlation coefficient [7].

Statistical analysis

All statistical analyses were performed using JMP version 15.0 (JMP, SAS Institute Inc., Cary, NC). Statistical significance was set to P=0.05. Association between percent change in SUV over the two year period and baseline BMI, age, coronary calcium score, cardiovascular risk factors, and blood chemistry were assessed using Spearman correlation analyses. Computed tomography derived values were quantified using Hounsfield units (HU). Hounsfield unit thresholds are determined by luminal contrast density in scans and can be used to assess plaque composition [15]. Association between the percent change in HU over the two year period and the same baseline parameters were tested using Spearman

correlation analysis.The same analyses were then repeated with stratification by gender.

Baseline and follow-up values of SUV and of HU were compared using paired Wilcoxon signed rank tests to determine if there was a significant increase over the two year period. The same analyses were then repeated with stratification by gender.

Paired Wilcoxon signed rank tests were used to determine if the percent change in SUV was greater than percent change in HU in an individual over the two year period. Correlation analyses were performed to determine if an individuals' change in SUV and change in HU were associated.

Results

All of the participants in the study had a host of baseline characteristics measured at the time of their initial PET/CT scan (Table 1). These characteristics included BMI, age, blood chemistry values, and cardiovascular risk factors. When subjects came in for their two year follow-up, BMI was again measured.

Percent change in SUVmean over the two year period correlated with multiple baseline characteristics. These were BM land systolic blood pressure. When stratified by gender, males had significant correlations of percent change in SUVmean with BMI (r=0.85, P<0.0001) and systolic blood pressure r= 0.65, P=0.0082) (Figure 1). However, there were no significant correlations between any female baseline characteristics and percent change in SUVmean. Percent change in HU did not correlate with BMI or systolic blood pressure when the whole group was considered and when stratified by gender.

Paired Wilcoxon signed rank tests in all subjects showed no significant difference between baseline and follow-up values for SUVmean (P=0.0770). The paired Wilcoxon signed rank tests for SUVmax (P=0.0491) and HU (P=0.0126) showed a significant decrease (Table 2). A box plot was derived of the baseline and follow-up values for SUVmean, SUVmax, and HU of all subjects (Figure 2). When stratified by gender, there were no significant differences for SUVmean (males, P=0.3591; females, P=0.1953), SUVmax (males, P=0.0919; females, P= 0.4435), and HU (males, P=0.1514; females, P=0.0547).

The change in SUVmean and the change in HU over the two year period for each individual were not correlated r= 0.31, P= 0.152). The change in SUVmean was significantly greater than the change in HU for an individual as determined by a Wilcoxon signed rank test (P=0.0016).

Both increases and decreases in SUVmean were found. This was visibly observable on the PET scans, as can be seen in Figure 3 of an overweight male with increased ^{18}F-NaF uptake and a male with a normal weight and decreased ^{18}F-NaF uptake.

Discussion

While we investigated correlations of change in SUVmean over a two year period with many risk factors, our study found that change in SUVmean over a two year period was positively correlated with BMI and blood pressure in males only. There was no correlation between change in SUVmax or change in HU and these baseline characteristics. There was no correlation between change in SUVmean and other baseline characteristics tested. There was no significant increase in SUVmean, SUVmax, or HU over the two year period. Percent change in SUV for an individual was significantly larger than percent change in HU for an individual.

Previous studies have shown associations between SUVmean and BMI [7, 11]. However, this is the first longitudinal study to show the significant correlation between the percent change in SUVmean over a short term and BMI. This illustrates that not only does the quantity of microcalcification correlate with BMI, but so does the rate of microcalcification. High BMI is a known risk factor for cardiovascular disease [16, 17]. Obesity is associated with a shorter lifespan as well as a greater proportion of life lived with cardiovascular disease [18]. As countries are experiencing economic growth, incidences of obesity and cardiovascular disease are also growing [16]. It will become increasingly important to understand how BMI can impact heart calcification. Our study confirms that there is a strong correlation between BMI and the rate of microcalcification in healthy subjects, and reveals ^{18}F-NaF as an effective tool for observing this, even over a period as short as two years. Our study included subjects whose BMI remained relatively the same, with the average change in BMI being -0.96±5.17%. Follow-up studies could be done on subjects in programs targeted for weight loss to see if the rate of microcalcification correlates with change in BMI.

With the strength of the association between change in SUVmean and BMI, it is unsurprising that blood pressure significantly correlated with change in SUVmean. There is a strong association between obesity and high blood pressure [19]. Fluorine-18-NaF emission tomography could be used in the future to see if the use of blood pressure lowering medications or diets could slow or even reverse the rate of microcalcification.

The correlations of percent change in SUV with BMI and blood pressurewere only significant for the males. This may be due to small sample size for the females (n=8). Additionally, when men and women who are free of cardiovascular disease at age 50, as were the healthy subjects in this study, are compared, men are significantly more likely to develop cardiovascular disease during the remainder of their lifetime [20]. This may impact the significant correlations between male characteristics and microcalcifcation in comparison to females. However, an examination of numerous studies provides a conflicting view of how sex impacts ^{18}F-NaF uptake. In a latitudinal study examining SUV with BMI, there were correlations in both males and females; however, these findings were less significant for females [7]. Furthermore, a study found an association between carotid ^{18}F-NaF uptake and male sex [12]. On the other hand, Bloomberg et al. (2017) found female sex an independent factor of increased coronary artery ^{18}F-NaF uptake in their study of healthy adults [11].

Before analyzing the data for this study, we expected that SUV and HU would have a significant increase over the two

Table 1. *Participant baseline characteristics and blood chemistry values.*

Subject Characteristics	Males (n = 15)	Females (n = 8)	P value
Weight (kg)	84.0 (79.5 - 89.3)	64.75 (59.80 - 66.28)	<0.0001
Height (cm)	176.5 (173 – 182)	163.75 (159.25 - 171.13)	0.0005
BMI (kg/m^2)	27.40 (25.45 - 29.06)	23.63 (22.31 - 25.66)	0.0074
Age (years)	52 (35 - 60)	51 (45.5 - 62.5)	0.1303
Systolic BP (mmHg)	130 (120.5 - 144.5)	124 (110 - 144.4)	0.1435
Diastolic BP (mmHg)	81.5 (73 - 83)	74.2 (64.6 - 85.1)	0.2122
Pulse Rate (beats/minute)	61 (54 - 77)	62.5 (60 - 70)	0.7549
Total Cholesterol (mmol/L)	4.9 (4.6 - 5.6)	5.35 (4.00 - 5.95)	0.8289
LDL cholesterol (mmol/L)	3.2 (3.0 - 3.9)	3.2 (2.08 - 4.13)	0.8103
HDL cholesterol (mmol/L)	1.1 (1 - 1.5)	1.55 (1.3 - 1.88)	0.0086
Triglycerides (mmol/L)	0.88 (0.65 - 1.34)	0.79 (0.58 - 1.00)	0.0842
Fasting Plasma Glucose (mmol/L)	5.9 (5.5 - 6.1)	5.3 (4.85 - 5.34)	0.0049
HbA1c (mmol/mol)	36 (33 - 37)	33 (29.75 - 35)	0.3604
CRP (mg/L)	1.0 (1.0 - 2.1)	1 (1 - 2.65)	0.4560
Fibrinogen (umol/L)	9.1 (8.2 - 10.1)	8.6 (7.9 - 9.65)	0.2303
White Blood Cell Count (cells/L)	6.1 (4.7 - 6.5)	5.3 (4.3 - 7.2)	0.2699
Creatinine (umol/L)	87 (82 - 94)	68 (64.5 - 74.3)	0.0021
eGFR (mL/min/1.73 m^2)	81 (73 - 87)	79.5 (73.3 - 83.8)	0.5825

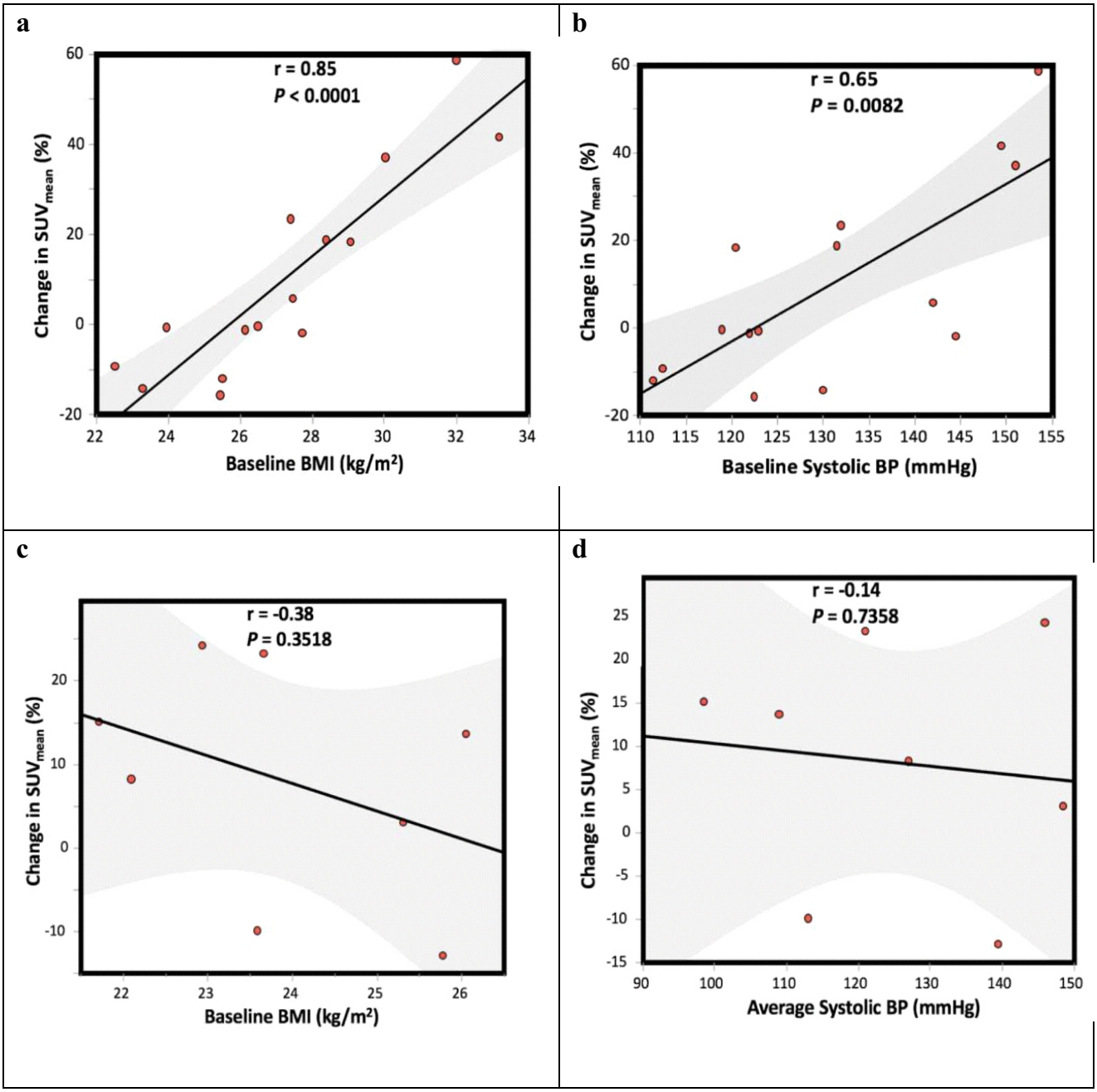

Figure 1. Change in SUVmean in males over a two year period significantly correlated with a)BMI and b)systolic blood pressure. Change in SUV in females over a two year period did not correlate with a) BMI or b)systolic blood pressure

Table 2. *The median and interquartile range of baseline and follow-up values for SUVmean, SUVmax, and HU.*

	Median Baseline	Median Follow-up	P-value
SUVmean	0.632 (0.554 - 0.726)	0.659 (0.595 - 0.802)	0.077
SUVmax	3.64 (2.71 - 4.42)	3.26 (2.67 - 3.69)	0.0491
HU	30.5 (28.1 - 32.6)	28.8 (27.7 - 29.6)	0.0126

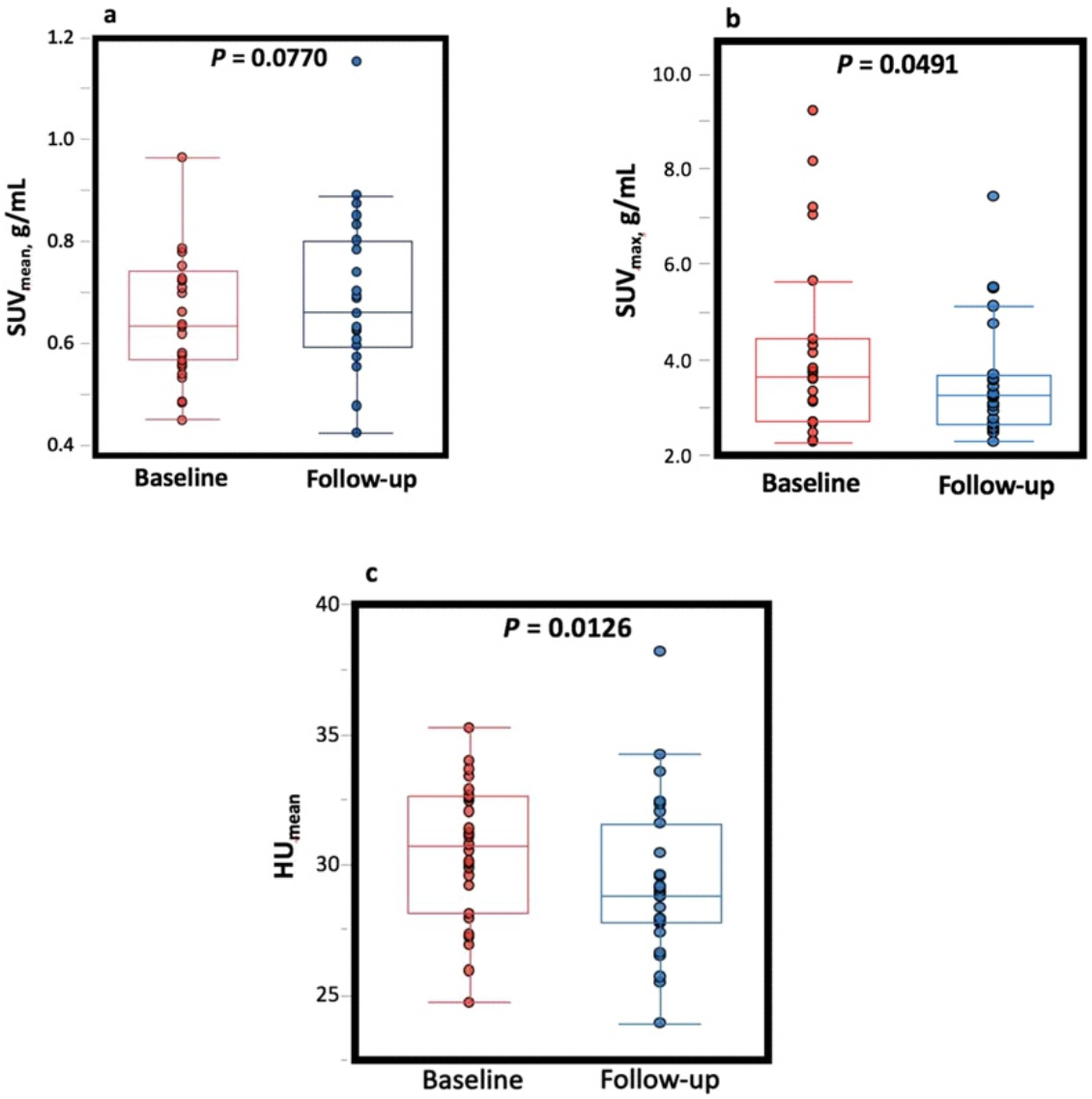

Figure 2. Boxplots of baseline and follow-up values of all subjects for a)SUVmean, b)SUVmax, and c) HU. P values are derived from nonparametric paired signed rank tests. Whiskers represent 25% and 75% quartiles.

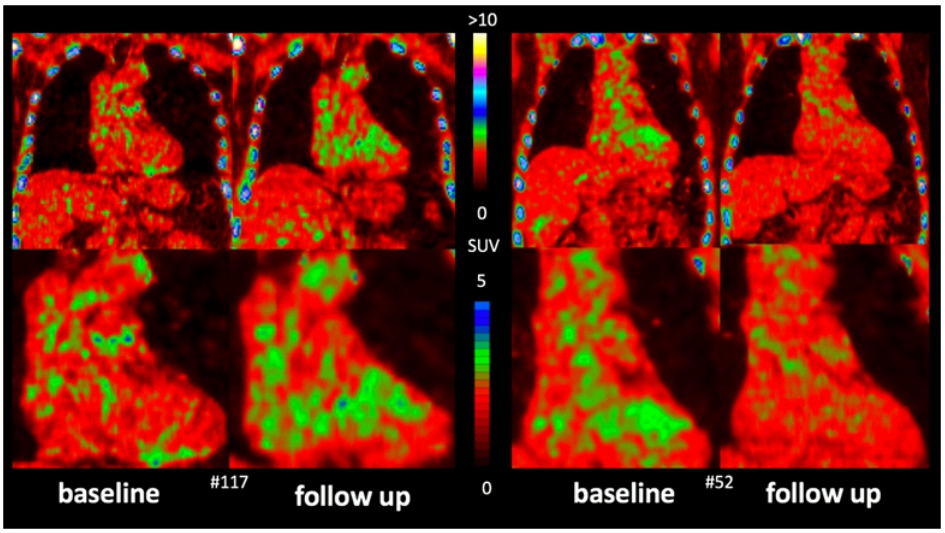

Figure 2. This figure shows baseline and follow-up PET scans of two individuals. The patient on the left (male, 65-year-old, BMI of 32.0kg/m^2) shows visibly increased ^{18}F-NaF uptake, suggesting atherosclerosis progression, and the patient on the right (male, 60-year-old, BMI of 22.5kg/m^2) shows decreased ^{18}F-NaF uptake, suggesting atherosclerosis regression. Pixel-wise SUV was reported as (mCi/g of tissue)/(mCi injected/body weight in g).

year interval as microcalcification progressed. This was not the case. This was likely because subjects had both increases and decreases of SUV and HU over the two year interval, suggesting the occurrence of both atherosclerosis progression and regression in healthy subjects.

The change in HU and SUV over the two year period did not correlate, despite both being ways to assess calcification in the coronary arteries. However, when a paired Wilcoxon signed rank was used the percent change in SUV was significantly greater than the percent change in HU. This is likely because ^{18}F-NaF has been shown to indicate the presence of microcalcifications before they are large enough to be visualized on a CT scan[1, 3]. Fluorine-18-NaF can also distinguish between micro and macrocalcification while values derived from CT only cannot [2].

Both Framingham risk score and calcium score did not correlate with change in SUVmean of ^{18}F-NaF over the two year period. Framingham heart score is intended to predict a 10 year risk. Studies have shown that the estimated 10 year risk for cardiovascular disease increases as ^{18}F-NaF uptake increases [11], yet the Framingham heart score did not correlate with an increase in ^{18}F-NaF suggesting it may not be a reliable indicator for progression of microcalcification in healthy subjects in this time frame. Our study used healthy participants, a majority of whom had a calcium score of 0. However, all of them had uptake of ^{18}F-NaF suggesting calcification occurring, and individuals with increased calcification over the two year period often had a calcium score of 0. Multiple studies have shown coronary calcium score as unreliable for detecting early signs of coronary artery disease in low risk subjects [21-23]. This study further indicates inefficacies of calcium score in low risk subjects to predict an increase in microcalcification over the short term.

Some limitations of our study have to be mentioned. This study was limited by the small sample size (n=23). This was especially limiting for the females (n=8). Furthermore, our study only included healthy subjects, and none of the subjects had any coronary events. This made us unable to see if microcalcification over the short term was more detectable in higher risk groups, and what factors correlated to coronary events. Furthermore, we did not use any software to register the baseline and follow-up images to each other. Instead, images were selected by hand and the whole heart region traced. However, our method had a high inter-operatory reproducibility value with minimal deviation. Images were all segmented by the same individual, with baseline and follow-up images observed in the same session to minimize variation.

In conclusion, CAD is the leading cause of death worldwide. This study was done on healthy subjects without a diagnosis of coronary artery disease, but progression of atherosclerosis was still visible using ^{18}F-NaF PET/CT imaging in the majority of subjects. While a large component of coronary artery disease is genetic, the risk of coronary events can be drastically reduced by lifestyle change [17]. Despite the limitations of a small sample size, our study showed that the rate of increase of microcalcification significantly correlates with BMI and blood pressure in males over a two year period. This reveals the predictive power of baseline characteristics and ^{18}F-NaF imaging in determining future calcification when Framingham heartscores and coronary artery calcium scores did not. Further longitudinal studies should be done with larger sample sizes to determine the utility of predicting microcalcification progression using baseline characteristics, as well as to determine the best ways to prevent progression and promote regression of atherosclerosis using this methodology.

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