18F-sodium fluoride PET/CT provides prognostic clarity compared to calcium and Framingham risk scoring when addressing whole-heart arterial calcification

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Abstract:

Aims

To investigate the benefit of utilizing 18 F-sodium fluoride (NaF) PET/CT over calcium and Framingham scoring for potential preventative coronary artery disease (CAD) intervention.

Methods and Results  This retrospective study included 136 participants (ages 21-75, BMI 18-43 kg/m²): 86 healthy controls and 50 patients. CT heart segmentations were superimposed onto PET images and standard uptake values (SUV) were calculated by a semi-auto segmentation method of drawing volumes of interest around the heart. Inter-group comparisons were made matching 37 patient/control pairs based on age, gender, and BMI. ROC curves were generated to determine how well SUV and Framingham methods predicted patient status. Regressions including all 136 participants were performed between SUV, age, and BMI.
Patients exhibited higher average SUV (SUV mean; \( P = .006 \)) and Framingham scores (\( P = .02 \)) than controls. However, ROC curves indicated that SUV mean could discriminate patients from controls (AUC = 0.63, \( P = .049 \)), but Framingham scores could not (AUC = 0.44, \( P = .38 \)). Calcium scores and maximum SUV (SUV max) did not differ between patients and controls. SUV mean correlated with age and BMI among females (age, partial \( R^2 = 0.16, P = .001 \); BMI, partial \( R^2 = 0.12, P = .004 \)) and males (age, partial \( R^2 = 0.28, P < .0001 \); BMI, partial \( R^2 = 0.22, P < .0001 \)).

Conclusion Unlike calcium scores, NaF PET/CT-derived values differed between patients and controls. Framingham risk score patterns echoed those of SUV mean, but were not sensitive enough to predict patient status. SUV mean values increased with age and BMI. Therefore, incorporation of NaF PET/CT into routine prognostic CAD assessment might prove beneficial for assessing early-stage plaque calcification in coronary arteries.

Keywords: atherosclerosis; coronary artery disease; PET/CT; 18 F-NaF; calcification

Response to Reviewers:

Response to Reviewer Comments
We thank the reviewers for the constructive comments and the editor for allowing us to revise the manuscript. We have addressed each comment as described below and revised the manuscript accordingly.

Reviewer #1: The present study appears promising, however the applied methodology is flawed. The study assessed whether NaF PET/CT is a better technique compared to the conventional CAC and Framingham risk score to identify patients vs healthy controls.

Far more interesting would be to determine the prognostic value of NaF PET compared to CAC and the Framingham risk score. Both the CAC and the Framingham risk score are prognostic scores. NaF PET is indeed not a technique for the detection of obstructive CAD, in other words not a diagnostic modality. The differentiation between symptomatic patients and healthy controls is not specific enough, since symptoms are not necessarily the results of myocardial ischemia. As such, the methodology is flawed. We agree with the reviewer that CAC and Framingham risk scores are in fact prognostic in nature. The weight and use of CAC and Framingham risk scoring will not be heavy in symptomatic patients, as clinicians will move towards more sensitive diagnostic methods of assessment for obstructive myocardial ischemia, such as CCTA and TEE.

NaF PET/CT perhaps could have merit as a prognostic or preventative methodology for assessing both future cardiovascular events and whole heart micro-calcifications due to lacking in structural sensitivity and localization due to low imaging resolution. We addressed the first comment, and have discussed the current use of NaF PET/CT in greater detail than previously addressed in the introduction, and commented on the fact that its limitations are preventative, and not a diagnostic tool for obstructive myocardial ischemia. Furthermore, we have strengthened the differentiation between patients and healthy controls with complete inclusion/exclusion criteria. Further details include:

- patients with chest pain were recruited from a population referred for a coronary CT-angiography. These patients were already documented to have angina, and CCTA suggests that these patients had symptoms associated with severe enough cardiovascular related issues to be recommended for further intervention. We make sure to clarify that referral for CCTA does not suggest obstructive myocardial ischemia, but it does propose calcified causation involving the arteries that is now symptomatic.
- exclusion of subjects with major history of cardiovascular events (i.e. acute myocardial infarction, transient ischemic attack, ischemic stroke), cancer, chronic inflammatory disease, or kidney failure. These patients are already at increased cardiovascular risk and are less likely to benefit from vascular calcification imaging with PET.
- Only patients with a 10-year risk for fatal cardiovascular disease equal to or above 1%, as estimated by the body mass index (kg/m2) based Systematic Coronary Risk Evaluation (SCORE) tool, were eligible for inclusion.
The discussion is not well written and confusing. Furthermore, a few statements made in the discussion are simply not true (Coronary computed tomography angiography (CCTA), X-ray angiography, and intravascular ultrasound are all methods that require anesthetics and are often only employed after the patient exhibits some acute angina or coronary event). CCTA does not require anesthetics and is not employed in patients with typical angina, patients with ACS or after a coronary event.

Thank you for this comment. A major change we made regarding this comment was altering the organization and wording of the discussion section to make it clearer. Below are the previous and current organizations of the discussion section:

- Organization
  - summarization of the results section
  - background biochemistry of how the radiotracer NaF interacts with calcium and osteoblastic metabolism within the bones and arteries
  - Reasons as to why we calculate and control for blood pooling SUV values within the heart
  - Further benefits of NaF and how this methodology will fit into the current way clinicians examine cardiovascular disease
  - Limitations of NaF imaging methodology
  - Concluding statement about NaF offering additional insight about the progression of CAD

- Discussion changes:
  - Organization
  - summary of results
  - Reasons as to why we calculate and control for blood pooling SUV values within the heart
  - Current literature of both prognostic and diagnostic tools to assess for coronary complications (including CAD)
  - Benefits of NaF and how this methodology will fit into the current way clinicians examine general heat/cardiovascular complications
  - Limitations of NaF imaging methodology and application

- The statement, (Coronary computed tomography angiography (CCTA), X-ray angiography….acute angina or coronary event.) Was changed to acknowledge that CCTA does not require anesthesia.

Reviewer #2:

First, the characterization of the patients is rather general. Additional information could be provided, for example:

1. What was the duration of angina? It would seem that a patient with stable angina for 2 years (perhaps due to a small lesion not suitable for stenting) might be a different population than patients with angina for two weeks.

We completely agree. Unfortunately, the duration of the angina is not known. Angina was present before patients were referred for a coronary CTA (the values or results of which we do not have in our retrospective data tables/files). We have added this fact as a limitation in the discussion section.

2. The study says that other cardiovascular disease was excluded, but how was this known?

"other" or severe cardiovascular disease was written as a portion of the exclusion criteria of the prospective CAMONA study, and has been described as follows:

Patients with chest pain were recruited from those referred for a coronary CT-angiography. Only patients with a 10-year risk for fatal cardiovascular disease equal to or above 1 %, as estimated by the body mass index (kg/m2) based Systematic COronary Risk Evaluation (SCORE) tool, were eligible for inclusion."

"We excluded subjects with a history of major cardiovascular events (i.e. acute myocardial infarction, transient ischemic attack, ischemic stroke), cancer, chronic inflammatory disease, or kidney failure. These patients are already at increased cardiovascular risk and are less likely to benefit from vascular calcification imaging with PET."
For example, I assume that the authors are distinguishing chest discomfort associated with mitral valve prolapse or a hypertrophic cardiomyopathy from angina. So presumably TTEs were performed in all subjects, both the angina and control groups to exclude these patients. This could be stated, since the description says no other cardiovascular disease was present in the control group. Presumably subjects with atrial fibrillation were excluded.

Subjects with atrial fibrillation were excluded, yes. TEEs were not performed on participants. Previous history of any cardiovascular events were criteria for exclusion.

3. Glycated hemoglobin and total cholesterol were reported, but how many patients in each group were being treated for type 2 diabetes? Did all the patients in the "non-pain" group have exercise testing?

Type II diabetes was an exclusion criterion for this study and has been clarified as such in the methodology “participant selection” section of the manuscript.

4. The description of the study design is unclear. It looks like all patients had coronary calcium scoring and 18F PET. But only the angina group had cardiac CT angiography, correct?

Yes, only the patient angina group had the cardiac CT angiography. Clarification has been made in the study design section of the manuscript.

So it is possible that patients with subclinical CAD were included in the healthy group. If this is the case, then the value of 18F PET is perhaps underestimated. It is possible that the healthy group did contain persons with sub-clinical calcium deposition in their arteries. Therefore, both the calcium scores and the SUV of the healthy control group could contain people with above 0 calcium in their coronary vasculature, increasing both of these averages. CAD is not usually diagnosed in asymptomatic individuals.

Second, the most interesting point would be increase 18F uptake in a noncalcified lesion detected by CT angiography. Can the authors provide some insight into the frequency of this finding?

Thank you for this comment. Unfortunately, we did not have access to the CT angiography data to correlate with PET uptake. 18F-NaF PET enables the assessment of coronary osteogenesis by interaction with or affinity to surface hydroxyapatite. Therefore, there might be lesions with micro-calcification that are visible on 18F-NaF PET but not detected via CT angiography labeled as “non-calcified” plaque. Furthermore, there could be inflammatory plaque types with some detection of SUV uptake as they begin to calcify.

Minor points
Page 5, line 27. The authors refer to coronary calcium score and the Framingham risk score as a functional assessment of coronary disease.

This error has been changed in the manuscript.

Page 8, line 11. Smoking habits were used to calculate Framingham risk score. But subjects with a smoking history were excluded.

This is a valid point. Framingham score calculated for an individual that does not smoke would be based on other risk factors, which we believe would be still useful for prognosis purposes.
Figures:

Figure 1

Volume of interest (VOI) capturing a representative heart. VOIs were thresholded to exclude voxels under -50 HU before being superimposed onto corresponding PET images.

Figure 2
Figure 2  
(a) Patients had significantly higher SUV\textsubscript{mean} than did healthy controls, but (b) there was no difference in SUV\textsubscript{max} between patients and controls. Midline represents mean, box represents SD, and whiskers represent range.

Figure 3  
Fused PET/CT example of (top) a 42-year-old male patient with a BMI of 36.1 kg/m\(^2\) and (bottom) the corresponding matched control, a 43-year-old male with a BMI of 32.9 kg/m\(^2\). The patient displays greater radiotracer uptake, indicated by colored pixel intensity.
Patients had significantly higher 10-year Framingham scores than did healthy controls. Midline represents mean, box represents SD, and whiskers represent range.
Figure 5  Patients and controls had similar (a) calcium masses, (b) calcium volumes, and (c) overall calcium scores. Midline represents mean, box represents SD, and whiskers represent range.
Figure 6  

SUV
\text{mean}, but not Framingham scores, accurately predicted patient status. SUV
\text{mean} data-driven optimal cut-off point: 0.76 g/mL. TPR = true positive rate, FPR = false positive rate, FRS = Framingham risk score.
Among females and males, both (a) age, adjusted for BMI and dosage, and (c) BMI, adjusted for age and dosage, were positively correlated with \( \text{SUV}_{\text{mean}} \). (b) Adjusted age was not correlated with \( \text{SUV}_{\text{max}} \) among females or males, while (d) adjusted BMI was positively correlated with \( \text{SUV}_{\text{max}} \) among males, but not females.
18F-Sodium Fluoride PET/CT Provides Prognostic Clarity Compared to Calcium and Framingham Risk Scoring when Addressing Whole-Heart Arterial Calcification

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“Conflict of Interest: none declared”
Abstract:

**Aims**

To investigate the benefit of utilizing $^{18}$F-sodium fluoride (NaF) PET/CT over calcium and Framingham scoring for potential preventative coronary artery disease (CAD) intervention.

**Methods and Results**

This retrospective study included 136 participants (ages 21-75, BMI 18-43 kg/m²): 86 healthy controls and 50 patients. CT heart segmentations were superimposed onto PET images and standard uptake values (SUV) were calculated by a semi-auto segmentation method of drawing volumes of interest around the heart. Intergroup comparisons were made matching 37 patient/control pairs based on age, gender, and BMI. ROC curves were generated to determine how well SUV and Framingham methods predicted patient status. Regressions including all 136 participants were performed between SUV, age, and BMI.

Patients exhibited higher average SUV ($SUV_{mean}$; $P = .006$) and Framingham scores ($P = .02$) than controls. However, ROC curves indicated that $SUV_{mean}$ could discriminate patients from controls (AUC = 0.63, $P = .049$), but Framingham scores could not (AUC = 0.44, $P = .38$). Calcium scores and maximum SUV ($SUV_{max}$) did not differ between patients and controls. $SUV_{mean}$ correlated with age and BMI among females (age, $partial R^2 = 0.16$, $P = .001$; BMI, $partial R^2 = 0.12$, $P = .004$) and males (age, $partial R^2 = 0.28$, $P < .0001$; BMI, $partial R^2 = 0.22$, $P < .0001$).

**Conclusion**

Unlike calcium scores, NaF PET/CT-derived values differed between patients and controls. Framingham risk score patterns echoed those of $SUV_{mean}$, but were not sensitive enough to predict patient status. $SUV_{mean}$ values increased with age and BMI. Therefore, incorporation of NaF PET/CT into routine prognostic CAD assessment might prove beneficial for assessing early-stage plaque calcification in coronary arteries.

**Keywords:** atherosclerosis; coronary artery disease; PET/CT; $^{18}$F-NaF; calcification
**Background**

Coronary Artery Disease (CAD) is a condition characterized by plaque deposition and luminal obstruction in the arteries of the heart. This can occur in the main coronary arteries: left coronary artery (LCA), right coronary artery (RCA), and left descending artery (LDA), or in the minor coronary arteries known as microcalcifications. The disease affects one third of women and one half of men in their lifetimes [1, 2]. An American will have a coronary event approximately every 25 seconds and one will die of one every minute [2]. Early prognostic assessment of this disease is therefore crucial to achieve adequate care and good quality of life [2-4].

Current prognostic modalities to assess coronary artery calcification include calcium scoring and Framingham risk scoring. Diagnostic methods include stress echocardiography, coronary CT angiography (CCTA), myocardial perfusion imaging utilizing single photon emission computed tomography (CT) or ultrasound, and cardiovascular magnetic resonance [5].

There are other ways of assessing CAD, but the methodologies listed above represent the main imaging modalities in current use. In addition, the structural methodology of coronary artery calcium scoring (CAC), along with life styles measurements and blood chemistry values, can be the primary factor when measuring risk in asymptomatic patients [6]. A lack of consensus exists when considering the strength of association between CAC scores and adverse coronary events [6, 7]. Some studies suggest CAC scores are appropriate for assessing individuals with intermediate CAD risk, but prove less beneficial when addressing those at low risk [6-8]. While CAC has been traditionally quantified using the Agatson scoring method and has prognostic value in large populations, it has several limitations. [6].
CAC scoring can be conducted in various ways. Agatston scoring involves using a weighted value assigned to the densest voxel of a given coronary artery. Density is measured in Hounsfield units and an Agatston density score of 1 corresponds with 130-199 HU, 2 with 200-299 HU, 3 with 300-399 HU, and 4 with 400-499 HU or greater [6]. The weighted score is then multiplied with the area (in mm) of the calcification. Mass and volume based measurements also exist where the actual and mass and volume are measured in the artery being examined [6, 7]. Given the “stepwise” classification of the density Agatston score, this method may not examine subtle changes to coronary artery calcification.

Framingham risk scoring is focused on assessing future coronary events. This method estimates the 10-year CAD risk of an individual on the basis of various criteria including cholesterol, age, gender, and systolic blood pressure [8, 9]. Framingham scoring uses variables known to increase CAD to determine the likelihood of a coronary event, but it does not provide physiological evidence of calcified plaque or predict how quickly CAD will progress.

Calcified plaque therefore presents a great challenge to clinicians and radiologists as numerous methods exist to analyze calcification for prevention and treatment purposes, all with differing strengths of association in determining risk for a coronary episode.

The exceedingly small scale of microcalcification lesions occupying arteries makes identification a serious challenge when using customary imaging techniques such as X-ray angiography, CT angiography, and calcium scoring. Using $^{18}$F-sodium fluoride (NaF) PET/CT imaging is a promising tool used in conjecture with other techniques; potentially assessing early coronary calcification because it allows for measurement of biologically active plaque in which
the quantity of radiotracer absorbed is directly correlated with the amount of calcified plaque
existent in the vasculature [9-16].

The purpose of our study was to investigate the usefulness of NaF standard uptake value
(SUV), as compared to calcium and Framingham risk scores; two methods heavily validated in
the literature as providing a strong predictive value of a coronary event [5-8]. Ideally, NaF will
serve as a biomarker of microcalcification in the coronary arteries for [1] identifying patients
with persistent chest pain and [2] quantifying age- and BMI-related increases in calcified plaque
content.

Materials and Methods

Our participants for this retrospective study were taken from the larger prospective
Cardiovascular Molecular Calcification Assessed by NaF PET/CT (CAMONA) study conducted
by Odense University Hospital (10-12, 17). The CAMONA study was approved by the Danish
National Health Committee on Health Research Ethics, registered at ClinicalTrials.gov
(NCT01724749) and conducted in accordance with the Declaration of Helsinki. All participants
provided written informed consent.

Participant Selection

The CAMONA study recruited participants for a prospective heart study. Of the 139 total
participants, 89 were designated as healthy controls and 50 were patients who had been referred
for coronary CT angiography on the basis of persistent chest pain. Angina in the patient
population was present before CCTA referral, but the duration of chest pain before referral is not
known. Three healthy controls did not have necessary PET/CT images on file for our analysis,
resulting in 86 control participants. Included in the study were 68 females (ages 21-75, BMI 18-37 kg/m²) and 68 males (ages 23-75 years, BMI 18-43 kg/m²).

Inclusion criteria for healthy controls included systolic blood pressure below 160 mm/Hg with diastolic blood pressure below 100 mm/Hg, glycated hemoglobin below 48 mmol/mol, and total serum cholesterol below 6.2 mmol/L. Patient groups were also required to meet this criterion in addition to the angina they experienced upon referral for angiography. Individuals with a history of smoking, and diagnosis of type II diabetes were not eligible for inclusion. Previous history of any cardiovascular events (i.e. acute myocardial infarction, transient ischemic attack, and ischemic stroke), cancer, chronic inflammatory disease, or kidney failure were eligible for exclusion. These patients are already at increased cardiovascular risk and are less likely to benefit from vascular calcification imaging with PET. Additionally, patients only patients with a 10-year risk for fatal cardiovascular disease equal to or above 1 %, as estimated by the body mass index (kg/m²) based Systematic Coronary Risk Evaluation (SCORE) tool, were eligible for inclusion.

All 136 participants were used to examine associative variables (i.e., age, BMI, SUV). Intergroup SUV comparisons—between patients and controls—utilized 37 pairs matched on the basis of age (± 6 years), gender, and BMI (± 3.2 kg/m²) to help minimize variables that could potentially influence CAD risk and manifestation. By controlling for these variables, we could directly compare diagnostic methods for detecting plaque deposition in similar cohorts with an already known diagnostic conclusion—whether they qualify as patients or controls.

Table 1. Comparison of Patients and Matched Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients ((n = 37))</th>
<th>Controls ((n = 37))</th>
<th>(P)-value</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Gender (No.)</th>
<th>Female = 18</th>
<th>Female = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male = 19</td>
<td>Male = 19</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.09</td>
<td>1.71 ± 0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.65 ± 16.02</td>
<td>77.49 ± 14.64</td>
</tr>
<tr>
<td>Dose (MBq)</td>
<td>171.19 ± 28.99</td>
<td>168.08 ± 37.56</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.19 ± 11.15</td>
<td>53.60 ± 9.88</td>
</tr>
</tbody>
</table>

*Note.*—*Unless otherwise specified, data are means ± SD. Patients did not differ from paired controls in gender, height, weight, dosage, or age.*

**Study Design**

NaF PET/CT imaging was performed on hybrid PET/CT machines under the same conditions for all individuals (GE Discovery STE, VCT, RX, and 690/710). Images were obtained 90 minutes after intravenous injection of 2.2 MBq of NaF per kg of body weight. CT images (140kV, 30-110 mA, noise index 25, 0.8 seconds per rotation, slice thickness 3.75 mm) were attenuation corrected. PET images were corrected for scatter and random coincidence.

Body weight, BMI, height, and age were also determined for all participants. Smoking habits, hypertension treatment, and blood analyses including fasting serum total cholesterol, LDL and HDL cholesterol, serum triglycerides, fasting glucose and glycated hemoglobin (HbA1c), and systolic blood pressure were measured to calculate a Framingham risk score for each...
participant. Comparison analyses were conducted between patients and controls to determine if these blood chemistry values were significant between groups.

**Table 2. Patient and Control Blood Chemistry Values**

<table>
<thead>
<tr>
<th>Average Blood Chemistry Levels</th>
<th>Control (n = 86)</th>
<th>Patient (n=51)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syst. BP (mmHg)</td>
<td>127.8 ± 17.04</td>
<td>131.1 ± 17.04</td>
<td>0.28</td>
</tr>
<tr>
<td>Diast. BP (mmHg)</td>
<td>76.7 ± 10.37</td>
<td>79.2 ± 7.79</td>
<td>0.10</td>
</tr>
<tr>
<td>Pulse (Beats/min.)</td>
<td>64.6 ± 12.63</td>
<td>64.7 ± 12.30</td>
<td>0.98</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.9 ± 0.86</td>
<td>5.3 ± 0.96</td>
<td>0.01*</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.1 ± 0.79</td>
<td>3.4 ± 0.88</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4 ± 0.45</td>
<td>1.4 ± 0.43</td>
<td>0.99</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.05 ± 0.65</td>
<td>1.2 ± 0.72</td>
<td>0.21</td>
</tr>
<tr>
<td>Homocysteine (umol/L)</td>
<td>8.8 ± 2.2</td>
<td>10.5 ± 3.99</td>
<td>0.01*</td>
</tr>
<tr>
<td>Fasting plasma Glucose (mmol/L)</td>
<td>5.5 ± 0.50</td>
<td>5.9 ± 0.87</td>
<td>0.01*</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>33.9 ± 4.10</td>
<td>37.4 ± 4.98</td>
<td>&lt; 0.0001 *</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.2 ± 2.98</td>
<td>2.6 ± 3.48</td>
<td>0.45</td>
</tr>
<tr>
<td>Fibrinogen (umol/L)</td>
<td>10.8 ± 10.7</td>
<td>10.2 ± 2.15</td>
<td>0.64</td>
</tr>
<tr>
<td>White Blood Cell Count (cells/L)</td>
<td>6.0 ± 1.80</td>
<td>6.4 ± 2.10</td>
<td>0.26</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>79.1 ± 13.12</td>
<td>82.8 ± 20.72</td>
<td>0.27</td>
</tr>
<tr>
<td>---------------------</td>
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<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>82.9 ± 13.10</td>
<td>75.8 ± 14.94</td>
<td>0.0069 *</td>
</tr>
</tbody>
</table>

*Note – Unless otherwise specified data are means ± SD. Patients differed from controls in Total Cholesterol, Homocysteine, Fasting Plasma Glucose, HbA1c, and eGFR.*

Calcium scores were determined by multiplying the area of calcium-containing pixels over 130 HU by weight (mg) to quantify the amount of calcified plaque. Regions of calcified plaque were identified by two radiologists.

**Quantitative Imaging Analysis**

SUV was calculated using the operator guided computer software PMOD (PMOD Technologies LLC, Switzerland). CT and PET images for each participant were uploaded onto the software and manipulated to create regions of interest (ROIs) on each CT image slice. This was done by segmenting regions around the heart on each 3.75mm thick coronal slice, moving anteriorly to posteriorly. These ROIs were then stacked to create 3D volumes of interest (VOIs). The VOIs encompassed only the vascular tissue of the heart—blood and the surrounding thoracic cavity were excluded by setting a threshold with a lower limit of -50 HU (Figure 1). The VOIs were generated using the CT images for structural clarity, then superimposed on the corresponding PET images. From here, average SUV (SUV\text{mean}) and maximum SUV (SUV\text{max}) were calculated within each VOI.

In order to control for potential alteration of SUV by circulating blood within the heart chambers, a singular ROI was generated in the superior vena cava (SVC). Anatomically, this ROI was automatically traced with a radius of 3.5mm near the base of the SVC, where it
connects to the right atrium (RA). A radius of 3.5mm was chosen to avoid capturing any of the
venous wall, which could also alter SUV. To control for blood pooling, SVC values were set as
controls in R version 3.5.1. statistical software when calculating regression analyses between
associating variables.

Statistical Analysis

Statistical significance was set at \( \alpha = .05 \). All analyses were performed using R version
3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

**Intergroup analyses.** Thirty-seven matched patient-control pairs were generated. Patients
and healthy controls were matched based on age, gender, and BMI to investigate differences in
SUV between individuals with persistent chest pain and their control counterparts. Comparative
analyses were run between these 37 matched pairs. Paired \( t \)-tests were conducted to determine
differences in SUV, Framingham scores, and calcium scores between patients and healthy
controls. For measures with significant differences between paired patients and controls, receiver
operator characteristic (ROC) curves were generated to determine how well these measures
predict patient status by plotting true positive rate against false positive rate. Areas under the
ROC curves (AUC) were calculated, then evaluated using one-sample Wilcoxon tests.

**Full set analyses.** Multiple regression analyses were performed to determine the
association between age and SUV, controlling for BMI, SVC, and dosage, as well as between
BMI and SUV, controlling for age, SVC, and dosage. Measures of both SUV\(_{\text{mean}}\) and SUV\(_{\text{max}}\)
were utilized. Similarly, regressions were performed using CT-derived values, as quantified
using HU. These regressions investigated the association between age and HU, controlling for
BMI and radiotracer dosage, as well as between BMI and HU, controlling for age and dosage.
Male and female regressions were conducted separately but final regression calculations included all 136 subjects.

Results

Intergroup Analyses

Participant characteristics. Patients did not differ substantially from paired controls in gender, height, weight, dosage, or age (Table 1).

Comparing patients to controls. Despite having similar HU values (0.88 HU lower among patients, CI: -3.05 – 1.30, \( P = .42 \)), compared to matched controls, patients had higher SUV\(_{\text{mean}}\) (0.09 g/mL higher among patients, CI: 0.03 – 0.15, \( P = .01 \)), but not SUV\(_{\text{max}}\) (0.04 g/mL lower among patients, CI: -1.06 – 0.98, \( P = .94 \); Figure 2), which was quite noticeable visually (Figure 3). Patients also had higher 10-year Framingham Risk Scores (2.45 higher among patients, CI: 0.46 – 4.45, \( P = .02 \); Figure 4). However, no differences were detected between patients and healthy controls on any calcium score measure (calcium mass: 58 mg lower among patients, CI: -481.93 – 365.93, \( P = .75 \); calcium volume: 65.71 mm lower among patients, CI: -458.34 – 326.91, \( P = .70 \); calcium score: 69 AU lower among patients, CI: -622.25 – 484.25, \( P = .77 \); Figure 5).

Although patients exhibited both higher Framingham scores and higher SUV\(_{\text{mean}}\) than controls, only SUV\(_{\text{mean}}\) (AUC = 0.63, \( u = 501.5 \), CI: -0.15 – -0.0003, \( P = .049 \)), and not Framingham scores (AUC = 0.44, \( u = 602 \), CI: -4.20 – 1.70, \( P = .38 \)), were able to predict patient status (Figure 6). The data-driven optimal SUV\(_{\text{mean}}\) cutoff point was determined to be 0.76 g/mL, such that healthy controls’ SUV\(_{\text{mean}}\) < 0.76 g/mL < patients’ SUV\(_{\text{mean}}\).
Full Set Analyses

Adjusted values of age, controlling for BMI, SVC, and radiotracer dosage, and adjusted values of BMI, controlling for age, SVC, and radiotracer dosage, were both positively correlated with SUV\textsubscript{mean} among females (age, partial $R^2 = 0.16$, $P = .001$; BMI, partial $R^2 = 0.12$, $P = .004$) and males (age, partial $R^2 = 0.28$, $P < .0001$; BMI, partial $R^2 = 0.22$, $P < .0001$), while SUV\textsubscript{max} only correlated with BMI among males (age, partial $R^2 = 0.001$, $P = .84$; BMI, partial $R^2 = 0.17$, $P = .001$) and did not correlate with anything among females (age, partial $R^2 = 0.01$, $P = .34$; BMI, partial $R^2 = 0.02$, $P = .27$; Figure 7). Furthermore, no interaction was found in either SUV model between age and gender (SUV\textsubscript{mean}, $\Delta R^2 = 0.08$, $P = .30$; SUV\textsubscript{max}, $\Delta R^2 = 0.01$, $P = .38$) or between BMI and gender (SUV\textsubscript{mean}, $\Delta R^2 = 0.07$, $P = .71$; SUV\textsubscript{max}, $\Delta R^2 = 0.02$, $P = .15$).

CT-derived HU, however, was not correlated with adjusted values of age among females (partial $R^2 = 0.03$, $P = .18$) or males (partial $R^2 = 0.0001$, $P = .94$), although adjusted BMI was negatively correlated with HU among females (partial $R^2 = 0.08$, $P = .03$) and males (partial $R^2 = 0.24$, $P < .0001$). As was the case in the SUV models, there was no interaction between age and gender ($\Delta R^2 = 0.02$, $P = .39$) or between BMI and gender ($\Delta R^2 = 0.01$, $P = .88$) within the HU models.

Reproducibility

To evaluate the validity of our methodology, 5 researchers conducted segmentation analysis using the methods described above for five study participants. The mean CV and ICC for the inter-operator reproducibility were 1.9% (1.4% to 2.3%) range, and 0.997%, respectively.
Discussion

Our study found that $SUV_{\text{mean}}$ was positively correlated with both age and BMI, two indicators of coronary artery calcification, among males and females. Compared to matched healthy controls, patients had significantly higher $SUV_{\text{mean}}$ and Framingham risk scores. There were no significant differences between matched patients and controls in any calcium scoring method (i.e., mass, volume, Agatston) or in $SUV_{\text{max}}$. The NaF PET/CT $SUV_{\text{mean}}$ ROC curve displayed a high degree of accuracy as a predictive method of coronary calcification, while the Framingham risk assessment score did not.

The only pattern detected by $SUV_{\text{max}}$ was a significant, positive correlation with BMI among males. This discrepancy between SUV measures is likely because CAD severity is determined more by the overall amount of plaque than by the metabolism of any one particular micro-calcification. Given the popularity of $SUV_{\text{max}}$ in NaF PET/CT atherosclerotic plaque detection, the sensitivity of this measure warrants further investigation.

Blood pooling in heart chambers could affect SUV values. Therefore, an ROI of the superior vena cava (SVC) was drawn manually, avoiding the venous wall, to calculate SUV of the blood. Blood SUV can either be included as a variable in multivariate linear regression or divided by coronary SUV directly to yield a target-to-background ratio (TBR). SVC SUV was utilized as a variable in the multivariate linear regression analyses in this study as it produced stronger correlation with age, BMI, and SUV associations. Further consideration of how blood circulation influences uptake values would likely result in a more accurate measure of SUV in anatomical vasculature.

Despite the use of prognostic modalities which serve as preventative assessments of coronary calcification and risk of a coronary events, there are still 1.5 million acute myocardial infarctions in the United States every year. Calcium scoring, along with X-ray and CT
angiography, are the methods most commonly utilized by clinicians in acute/emergency settings to assess coronary artery plaque manifestation [5, 18]. Often, calcification assessments occur after patients exhibit severe symptoms, such as chest pain, and are being assessed for revascularization [18]. However, coronary artery plaque and subsequent coronary artery disease (CAD) is often asymptomatic for years and requires a method to analyze small changes in plaque manifestation. Calcium Scoring has been validated in some studies as sufficient for determining risk in asymptomatic patients [6, 7, 18-22], yet limitations still exist. Agatston scoring is not conducive to radiation dose reduction which reconstructs images to reduce noise with changing voltage (kVp) and current measurements (mA). This reconstruction will alter pixel dimensions and can cause Agatston miscalculations of 3% to 31% [18]. Functional imaging such as SPECT and MR perfusion tests are beneficial for patients that require prolonged diagnostic intervention and monitoring, but the addition of NaF PET/CT as a methodology for monitoring coronary calcification over an extended period of time could prove beneficial. This is because, unlike functional imaging, which examines hemodynamic changes in response to stress, NaF measures subtle changes in the amount of calcium in the arteries [20-25].

Many of the procedures that diagnose and monitor coronary plaque deposition are invasive and are often limited to acute coronary events [1, 18, 20]. Coronary computed tomography angiography (CCTA), Xray angiography, and intravascular ultrasound monitor the amount of calcification in the vessels, and are therefore often a better assessment of disease burden than of near coronary event risk [18]. Arterial stenosis does not correlate with risk of an event. Single-photon emission computed tomography (SPECT) is a functional assessment to determine how the heart responds with added stressors, often exercise or an adenosine injection to mimic exercise’s effects. No quantification of plaque in the arteries results from this method. The benefits of $^{18}$F-
NaF PET/CT imaging in the context of current available imaging modalities is that it provides a way for clinicians to quantify subtle changes in plaque deposition that are not visible to the human eye (picomolar tissue concentrations). Early vascular calcification (microcalcification) in response to inflammation can be detected by NaF below CT resolution [26-31]. It is a method that is less invasive than an angiography. NaF PET/CT imaging utilizes a biochemical method and offers quantitative measurement of calcified plaque in the coronary arteries [10-12, 17, 32-34]. This method bypasses the need for subjective recognition with the naked eye by a radiologist, which may explain why calcium scores did not differ between patients and controls [12, 17]. Our methodology for calculating SUV only relied on the eyes of the technician for gross anatomic identification. A preliminary circle was drawn around the heart with the only requirement being that no outside bone or soft tissue be present in the VOI. Then, the VOI was automatically segmented using PMOD software to include HU values that were above a specific threshold of -50 HU. Even computer-derived CT values obtained from this segmentation did not differ between patients and controls or correlate with age or BMI. This suggests that NaF’s utilization of a functional pathway renders it sensitive to the formation of microcalcification long before it becomes detectable calcification.

NaF allows for early detection of atherosclerotic plaque because it exchanges a fluoride ion with a hydroxyl group of the accumulated hydroxyapatite in the coronary arteries [35, 19]. SUV$_{\text{mean}}$ is effective regardless of whether the plaque is visible to the human eye, or even to computer software, which makes it a promising tool, since coronary plaque can be clinically asymptomatic for years [35].

NaF elucidates functional pathways and is highly sensitive to small changes in plaque accumulation over short periods of time, which makes it ideal for monitoring medication
response. However, it has low spatial resolution [35], so it can be difficult to diagnose the loci of plaque deposition accurately in the small coronary arteries. Additionally, NaF is a radiotracer that is best absorbed by bone or “bone-like” material, so atherosclerosis due to an inflammatory response consisting of macrophage infiltration and thrombus generation would not be detected until the plaque becomes calcified [2, 34]. In the future, pairing NaF with FDG, a radiotracer that metabolically interacts with inflammation, could therefore prove beneficial to measure various types of plaque in the arteries so as to obtain a more holistic image of a patient’s health.

The limitations of our study include usage of fused PET/CT images previously provided by the scanner without additional registration to account for fusion error. This could potentiate miscalculations in SUV if CT and PET images do not line up exactly. However, our methodology allows for such a large VOI calculation of the entire heart, in comparison to segmenting individual arteries, potential shifts in fused images would not result in great SUV delineation. In addition, our methodology utilizes a semi-automated method and still relies partially on an operator that draws an initial VOI around the heart. Despite this, our method has a high inter-operator reproducibility value with minimal deviation in SUV calculations between operators. This study utilizes the CAMONA cohort where neither patients nor healthy controls had any prior history of heart disease, with patients initially being referred for angiography based on chest pain. It is likely that the cohort does not represent the range of SUV present in individuals with severe/ long-term heart disease. This fact does not change the fact the NaF PET/CT SUV was still able to detect subtle changes in micro-calcifications in the coronary arteries. The duration of the angina of the individuals in the patient cohort before recruitment to the CAMONA study is not known. Therefore, varying levels of plaque accumulation and risk of cardiac events between individuals is likely.
CAD is the leading cause of death worldwide, contributing to a third of all deaths among individuals over the age of 35 [36]. The disease itself is multifaceted in that it has both lifestyle and genetic causes, and it tends to be accompanied by various comorbidities that could enhance its progression [35-38]. NaF PET/CT imaging offers additional insight of the progression of coronary plaque and subsequent disease. Incorporating this function-based prognostic imaging modality into a clinical setting could expand awareness of patient health and pave the way for advances in care.
Declarations:

Ethics Approval and Consent to Participate
- not applicable

Consent for publication
- The CAMONA study was approved by the Danish National Health Committee on Health Research Ethics, registered at ClinicalTrials.gov (NCT01724749) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Availability of Data and Material
- The datasets used and analyzed in the current study are from the CAMONA cohort, and are available from the corresponding author on reasonable request.

Competing Interests
- The authors declare that they have competing interests.

Funding
- This work was not supported by any outside funding. The CAMONA files were retrospectively used and were already in the lab’s database system.

Author Contributions
- Olivia Sorci has contributed to a large portion of the data analysis and writing.
- Alexandra Batzdorf has contributed to a large portion of the statistical analysis and writing.
- Michael Mayer has contributed to a large portion of the writing.
- Sylvia Rhodes, Matthew Peng, Amanda Jankelovits, and Julia Hornyak contributed to a large portion of the data analysis and interpretation of data.
- Oke Gerke and Poul Flemming Høilund-Carlsen contributed to statistical advising of this manuscript.
- Abass Alavi and Chamith Rajapakse contributed to the methodological advising of this manuscript.

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“Conflict of Interest: none declared”
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Response to Reviewer Comments

We thank the reviewers for the constructive comments and the editor for allowing us to revise the manuscript. We have addressed each comment as described below and revised the manuscript accordingly.

Reviewer #1: The present study appears promising, however the applied methodology is flawed. The study assessed whether NaF PET/CT is a better technique compared to the conventional CAC and Framingham risk score to identify patients vs healthy controls. Far more interesting would be to determine the prognostic value of NaF PET compared to CAC and the Framingham risk score. Both the CAC and the Framingham risk score are prognostic scores. NaF PET is indeed not a technique for the detection of obstructive CAD, in other words not a diagnostic modality. The differentiation between symptomatic patients and healthy controls is not specific enough, since symptoms are not necessarily the results of myocardial ischemia. As such, the methodology is flawed.

We agree with the reviewer that CAC and Framingham risk scores are in fact prognostic in nature. The weight and use of CAC and Framingham risk scoring will not be heavy in symptomatic patients, as clinicians will move towards more sensitive diagnostic methods of assessment for obstructive myocardial ischemia, such as CCTA and TEE.

NaF PET/CT perhaps could have merit as a prognostic or preventative methodology for assessing both future cardiovascular events and whole heart microcalcifications due to lacking in structural sensitivity and localization due to low imaging resolution. We addressed the first comment, and have discussed the current use of NaF PET/CT in greater detail than previously addressed in the introduction, and commented on the fact that its limitations are preventative, and not a diagnostic tool for obstructive myocardial ischemia. Furthermore, we have strengthened the differentiation between patients and healthy controls with complete inclusion/exclusion criteria. Further details include:

- patients with chest pain were recruited from a population referred for a coronary CT-angiography. These patients were already documented to have angina, and CCTA suggests that these patients had symptoms associated with severe enough cardiovascular related issues to be recommended for further intervention. We make sure to clarify that referral for CCTA does not suggest obstructive myocardial ischemia, but it does propose calcified causation involving the arteries that is now symptomatic.
- exclusion of subjects with major history of cardiovascular events (i.e. acute myocardial infarction, transient ischemic attack, ischemic stroke), cancer, chronic inflammatory disease, or kidney failure. These patients are already at increased cardiovascular risk and are less likely to benefit from vascular calcification imaging with PET.
- Only patients with a 10-year risk for fatal cardiovascular disease equal to or above 1%, as estimated by the body mass index (kg/m2) based Systematic Coronary Risk Evaluation (SCORE) tool, were eligible for inclusion.
The discussion is not well written and confusing. Furthermore, a few statements made in the discussion are simply not true (Coronary computed tomography angiography (CCTA), X-ray angiography, and intravascular ultrasound are all methods that require anesthetics and are often only employed after the patient exhibits some acute angina or coronary event). CCTA does not require anesthetics and is not employed in patients with typical angina, patients with ACS or after a coronary event.

Thank you for this comment. A major change we made regarding this comment was altering the organization and wording of the discussion section to make it clearer. Below are the previous and current organizations of the discussion section:

- **Organization**
  - summarization of the results section
  - background biochemistry of how the radiotracer NaF interacts with calcium and osteoblastic metabolism within the bones and arteries
  - Reasons as to why we calculate and control for blood pooling SUV values within the heart
  - Further benefits of NaF and how this methodology will fit into the current way clinicians examine cardiovascular disease
  - Limitations of NaF imaging methodology
  - Concluding statement about NaF offering additional insight about the progression of CAD
- **Discussion changes:**
  - Organization
    - summary of results
    - Reasons as to why we calculate and control for blood pooling SUV values within the heart
    - Current literature of both prognostic and diagnostic tools to assess for coronary complications (including CAD)
    - Benefits of NaF and how this methodology will fit into the current way clinicians examine general heat/cardiovascular complications
    - Limitations of NaF imaging methodology and application
  - The statement, (Coronary computed tomography angiography (CCTA), X-ray angiography….acute angina or coronary event.) Was changed to acknowledge that CCTA does not require anesthesia.

Reviewer #2:

First, the characterization of the patients is rather general. Additional information could be provided, for example:

1. What was the duration of angina? It would seem that a patient with stable angina for 2 years
(perhaps due to a small lesion not suitable for stenting) might be a different population than patients with angina for two weeks.

We completely agree. Unfortunately, the duration of the angina is not known. Angina was present before patients were referred for a coronary CTA (the values or results of which we do not have in our retrospective data tables/files). We have added this fact as a limitation in the discussion section.

2. The study says that other cardiovascular disease was excluded, but how was this known?

“other” or severe cardiovascular disease was written as a portion of the exclusion criteria of the prospective CAMONA study, and has been described as follows:

Patients with chest pain were recruited from those referred for a coronary CT-angiography. Only patients with a 10-year risk for fatal cardiovascular disease equal to or above 1 %, as estimated by the body mass index (kg/m2) based Systematic COronary Risk Evaluation (SCORE) tool, were eligible for inclusion.”

“We excluded subjects with a history of major cardiovascular events (i.e. acute myocardial infarction, transient ischemic attack, ischemic stroke), cancer, chronic inflammatory disease, or kidney failure. These patients are already at increased cardiovascular risk and are less likely to benefit from vascular calcification imaging with PET.”

For example, I assume that the authors are distinguishing chest discomfort associated with mitral valve prolapse or a hypertrophic cardiomyopathy from angina. So presumably TTEs were performed in all subjects, both the angina and control groups to exclude these patients. This could be stated, since the description says no other cardiovascular disease was present in the control group. Presumably subjects with atrial fibrillation were excluded.

Subjects with atrial fibrillation were excluded, yes. TEEs were not performed on participants. Previous history of any cardiovascular events were criteria for exclusion.

3. Glycated hemoglobin and total cholesterol were reported, but how many patients in each group were being treated for type 2 diabetes? Did all the patients in the "non-pain" group have exercise testing?

Type II diabetes was an exclusion criterion for this study and has been clarified as such in the methodology “participant selection” section of the manuscript.

4. The description of the study design is unclear. It looks like all patients had coronary calcium scoring and 18F PET. But only the angina group had cardiac CT angiography, correct?
Yes, only the patient angina group had the cardiac CT angiography. Clarification has been made in the study design section of the manuscript.

So it is possible that patients with subclinical CAD were included in the healthy group. If this is the case, then the value of 18F PET is perhaps underestimated.

It is possible that the healthy group did contain persons with sub-clinical calcium deposition in their arteries. Therefore, both the calcium scores and the SUV of the healthy control group could contain people with above 0 calcium in their coronary vasculature, increasing both of these averages. CAD is not usually diagnosed in asymptomatic individuals.

Second, the most interesting point would be increase 18F uptake in a noncalcified lesion detected by CT angiography. Can the authors provide some insight into the frequency of this finding?

Thank you for this comment. Unfortunately, we did not have access to the CT angiography data to correlate with PET uptake. 18F-NaF PET enables the assessment of coronary osteogenesis by interaction with or affinity to surface hydroxyapatite. Therefore, there might be lesions with micro-calcification that are visible on 18F-NaF PET but not detected via CT angiography labeled as “non-calcified” plaque. Furthermore, there could be inflammatory plaque types with some detection of SUV uptake as they begin to calcify.

Minor points
Page 5, line 27. The authors refer to coronary calcium score and the Framingham risk score as a functional assessment of coronary disease.

This error has been changed in the manuscript.

Page 8, line 11. Smoking habits were used to calculate Framingham risk score. But subjects with a smoking history were excluded.

This is a valid point. Framingham score calculated for an individual that does not smoke would be based on other risk factors, which we believe would be still useful for prognosis purposes.