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(1) Title: Impact of Personal Characteristics and Technical Factors on Quantification of Sodium ^{18}F-Fluoride Uptake in Human Arteries: Prospective Evaluation of Healthy Subjects

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ABSTRACT

Sodium ^{18}F-fluoride (^{18}F-NaF) PET/CT imaging is a promising imaging technique for assessment of atherosclerosis, but is hampered by a lack of validated quantification protocols. Both personal characteristics and technical factors can affect quantification of arterial ^{18}F-NaF uptake. This study investigated if blood activity, renal function, injected dose, circulating time, and PET/CT system affect quantification of arterial ^{18}F-NaF uptake.

Methods: Eighty-nine healthy subjects were prospectively examined by ^{18}F-NaF PET/CT imaging. Arterial ^{18}F-NaF uptake was quantified at the level of the ascending aorta, aortic arch, descending thoracic aorta, and coronary arteries by calculating the maximum ^{18}F-NaF activity (NaFmax), the maximum target-to-background ratio (TBRmax/mean), and the maximum blood subtracted ^{18}F-NaF activity (bsNaFmax). Multivariable linear regression assessed the effect of personal characteristics and technical factors on quantification of arterial ^{18}F-NaF uptake.

Results: NaFmax and TBRmax/mean were dependent on blood activity (β = .34 to .44, P < .001 and β = -.68 to -.58, P < .001, respectively) and PET/CT system (β = -.80 to -.53, P < .001 and β = -.80 to -.23, P < .031, respectively). bsNaFmax depended on PET/CT system (β = -.91 to -.57, P < .001), but not blood activity. This finding was observed at the level of the ascending aorta, aortic arch, descending thoracic aorta, as well as the coronary arteries. In addition to blood activity and PET/CT system, injected dose affected quantification of arterial ^{18}F-NaF uptake, whereas renal function and circulating time did not.

Conclusion: Prospective evaluation of 89 healthy subjects demonstrated that quantification of arterial ^{18}F-NaF uptake is affected by blood activity, injected dose, and

PET/CT system. Therefore, blood activity, injected dose, and PET/CT system should be taken into account to generate accurate estimates of arterial ^{18}F-NaF uptake.

Keywords: PET/CT, sodium ^{18}F-fluoride (^{18}F-NaF), atherosclerosis, vascular calcification, quantification

INTRODUCTION

Sodium ^{18}F-fluoride (^{18}F-NaF) positron emission tomography/computed tomography (PET/CT) is a promising non-invasive imaging technique for assessment of atherosclerosis. ^{18}F-NaF PET/CT targets the active exchange of fluoride with hydroxyl ions of hydroxylapatite crystals producing fluorapatite (*1*). This process is believed to represent calcification metabolism of osseous tissue, including vascular calcification (*2-4*). By imaging vascular calcification metabolism, ^{18}F-NaF PET/CT can potentially identify patients at high cardiovascular risk (*4*) and improve cardiovascular risk stratification (*5,6*).

Although ^{18}F-NaF PET/CT imaging of vascular calcification is promising, implementing ^{18}F-NaF PET/CT in research and clinical settings is hampered by a lack of validated and standardized quantification protocols. Most studies quantify arterial ^{18}F-NaF uptake as the ratio between arterial wall and blood ^{18}F-NaF activity, known as the target-to-background ratio (TBRmax/mean). However, this method has been criticized to be too dependent on blood activity (*6*). In addition to blood activity, quantification of arterial ^{18}F-NaF uptake can be affected by personal characteristics and technical factors, including body weight, body surface area, renal function, injected ^{18}F-NaF dose, ^{18}F-NaF circulating time, and PET/CT system. It is not known which factors affect quantification of arterial ^{18}F-NaF uptake. Standardized and unbiased quantification of arterial ^{18}F-NaF uptake is imperative for both research and clinical settings, being a prerequisite for generation of reference values for arterial ^{18}F-NaF uptake with healthy aging, for response evaluation requiring repeat ^{18}F-NaF PET/CT examinations, and to allow for comparison of quantitative imaging results among studies.

The purpose of this study was to determine the effect of personal characteristics and technical factors on quantification of arterial ^{18}F-NaF uptake. By studying these effects in a group of healthy subjects we aimed to generate accurate estimates of arterial ^{18}F-NaF uptake. Secondary aims were to elucidate the effects of quantification methods on estimates of arterial ^{18}F-NaF uptake, to determine the optimal location for assessment of blood activity, and, finally, to evaluate rater reliability and agreement.

MATERIALS AND METHODS

This study is part of the "Cardiovascular Molecular Calcification Assessed by ^{18}F-NaF PET/CT" (CAMONA) study. CAMONA was approved by the Danish National Committee on Health Research Ethics, registered at ClinicalTrials.gov (NCT01724749), and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study subjects.

Subject Selection

Healthy subjects were prospectively recruited from the general population by local advertisement or from the blood bank of Odense University Hospital, Denmark. Subjects free of oncologic disease, autoimmune disease, immunodeficiency syndromes, alcohol abuse, illicit drug use, (symptoms suggesting) cardiovascular disease, or prescription medication were considered healthy and were eligible for inclusion. Pregnant women were not considered for inclusion. Healthy subjects were recruited to limit bias from cardiovascular disease on study results. Subjects were preselected by sex and age to secure a balanced inclusion of males and females aged 20–29, 30–39, 40–49, 50–59, > 60 years. This allowed us to study a wide range of subjects to ensure translation of our findings to various settings.

Study Design

Healthy subjects were evaluated by blood pressure measurements, blood analyses, ^{18}F-NaF PET/CT imaging, and non-contrast enhanced cardiac CT imaging. Blood pressure measurements were performed thrice after a supine rest of at least 30 minutes. The average of the last two measurements determined the systolic and diastolic blood pressure. Blood

analyses included fasting serum total cholesterol, serum LDL cholesterol, serum HDL cholesterol, serum triglycerides, fasting plasma glucose, glycated hemoglobin (HbA1c), serum creatinine, the latter being used to calculate the Modification of Diet and Renal Disease (MDRD) estimated glomerular filtration rate (eGFR). Furthermore, body weight, body height, body mass index, and body surface area according to Du Bois were determined. ^{18}F-NaF PET/CT imaging was performed on integrated PET/CT systems (GE Discovery 690/710, STE, VCT, and RX) at the PET center of Odense University Hospital, Denmark. Subjects were allocated to a PET/CT system at the discretion of the department's booking system. PET/CT system specifications and image reconstruction parameters are summarized in supplementary table 1. Each subject underwent PET/CT imaging at approximately 90 minutes after intravenous injection of approximately 2.2 MBq of ^{18}F-NaF per kilogram of body weight (*6*). The emission acquisition duration per bed position was 2.5 minutes. Total body PET images were acquired in 3D–mode and reconstructed into coronal, axial, and sagittal planes by an ordered subsets expectation maximization algorithm (GE VUE Point). PET images were corrected for attenuation, scatter, random coincidences, and scanner dead time. Low–dose CT imaging (140 kV, 30-110 mA, noise index 25, 0.8 seconds per rotation, slice thickness 3.75 mm) was performed for attenuation correction and anatomic orientation. To determine the coronary calcium score, non-contrast enhanced, breath-hold, cardiac CT imaging (120 kV, 100 mA, 0.4 seconds per rotation, slice thickness 2.5 mm) was performed with electrocardiogram gating at 50 % of the R–R interval. The effective radiation dose received for the entire imaging protocol was approximately 11 mSv.

Quantitative Image Analyses

All images were analyzed by version 4.0 of the Philips IntelliSpace Portal client. The image analyst was masked to subject demographics and PET/CT system specifications. For each subject, uptake of ^{18}F-NaF was determined in the ascending aorta, aortic arch, descending thoracic aorta, and coronary arteries according to previously published methods (*6*). In summary, for the coronary arteries, we manually drew a free-hand region of interest (ROI) around the cardiac silhouette on every slice of the axially oriented PET/CT images. We carefully excluded ^{18}F-NaF activity originating from bone tissue and cardiac valves by eliminating these areas from the ROI. For the aorta, we manually drew a free-hand ROI around the outer perimeter of the artery on every slice of the axially oriented PET/CT images. We carefully excluded skeletal-derived ^{18}F-NaF activity by eliminating these areas from the ROI. Per ROI, the maximum radiotracer-decay corrected ^{18}F-NaF activity (kBq/mL) was determined. Per arterial bed, maximum values obtained per ROI were summed and divided by the number of ROIs resulting in a single averaged maximum value (NaFmax) for the ascending aorta, aortic arch, descending thoracic aorta, and coronary arteries, respectively. Blood ^{18}F-NaF activity was determined in the lumen of the right atrium, aortic arch, right and left internal jugular vein, superior and inferior vena cava, and right and left femoral vein. Blood ^{18}F-NaF activity was determined by drawing a single ROI in the center of each vessel (or atrium) and was quantified as the radiotracer-decay corrected mean ^{18}F-NaF activity (bloodNaFmean). Quantification of blood ^{18}F-NaF activity is summarized in supplementary figure 1. To correct for blood ^{18}F-NaF activity, NaFmax was divided and subtracted by bloodNaFmean, respectively, to generate the maximum target-to-background ratio (TBRmax/mean) and maximum blood subtracted ^{18}F-NaF activity (bsNaFmax). Blood

pool correction was performed with superior vena cava bloodNaFmean only, because this location was least subject to spillover activity from adjacent ^{18}F-NaF avid structures. In addition, super vena cava bloodNaFmean could be determined with excellent inter- and intra-rater agreement (figure 2, supplementary tables 2 and 3). Quantification of arterial ^{18}F-NaF uptake is summarized in figure 1. The coronary calcium score, obtained from the cardiac CT images, was quantified in arbitrary units according to Agatston and as a volumetric score (mm^3) (*7*).

Rater Reliability and Agreement

Inter- and intra-rater reliability and agreement of NaFmax and bloodNaFmean were assessed two months after the initial analysis in a randomly selected sample of 10 subjects. Raters were masked for subject demographics, imaging specifications, and results from the initial analysis.

Statistical Analyses

Subject demographics were summarized by descriptive statistics. Mean bloodNaFmean was compared among vascular beds by the repeated measures one-way analysis of variance (ANOVA). Multivariable linear regression assessed the dependence of bloodNaFmean, NaFmax, TBRmax/mean, and bsNaFmax on personal characteristics and technical factors. We did not evaluate non-linear or interaction effects. First, we tested if the assumptions of no multicollinearity (tolerance statistic), independent errors (Durbin-Watson statistic), and homoscedasticity (graphically) between predictor variables were met. The assumption of no multicollinearity was violated by our predictor variables. Multicollinearity existed between injected dose, body weight, and body surface area (supplementary figure 2). The

issue of multicollinearity was resolved by removing body weight and body surface area from the regression analyses. Subsequently, stepwise selection of variables, based on Akaike's information criterion, was performed by a backward elimination strategy. The categorical variable "PET/CT system" was entered as factor into the model with the GE Discovery 690/710 as reference system. Variables not selected by the model were considered not related to arterial ^{18}F-NaF uptake. Variability in variable selection was evaluated and adjusted for by a bootstrap of 2,000 samples (*8*). Rater reliability was assessed by the intra-class correlation coefficient (ICC) (two-way random effects model assessing absolute agreement of single measures) (*9*). Rater agreement was assessed by calculation of the 95 % limits of agreement according to Bland and Altman (*10*). A two-tailed *P* value below .05 was regarded statistically significant. To internally validate our results, *P* values and 95 % confidence intervals were determined by a bootstrap of 2,000 samples. The sample size was based on the regression analysis. For every predictor variable (i.e. six continuous and one categorical variable) we aimed to include 10 subjects resulting in a sample size of 90 subjects. Statistical analyses were performed by statistical software R version 3.1.2 combined with the packages 'bootStepAIC' version 1.2-0, 'MASS' version 7.3-35, 'car' version 2.0-22, and 'QuantPsyc' version 1.5.

RESULTS

Between November 2012 and May 2014 we prospectively recruited 90 healthy subjects. One subject was excluded from the analysis because she refused the PET/CT examination due to claustrophobia. Subject demographics are summarized in table 1.

bloodNaFmean was significantly different among vessel beds (F = 66.6, P < .001) (figure 2). In particular, left internal jugular vein bloodNaFmean was up to 58 % higher than bloodNaFmean in other vascular beds (t = 10.2; P < .001). Similarly, right internal jugular vein bloodNaFmean was up to 39 % higher than bloodNaFmean in other vascular beds (t = 12.0; P < .001). Smaller, yet statistically significant, differences were observed between bloodNaFmean in the right atrium, aortic arch, superior vena cava, inferior vena cava, and femoral veins. Subsequent analyses were performed with superior vena cava bloodNaFmean only, because superior vena cava bloodNaFmean was least subject to spillover activity from adjacent ^{18}F-NaF avid structures and demonstrated excellent rater agreement (figure 2, supplementary tables 2 and 3). Superior vena cava bloodNaFmean significantly depended on injected dose and PET/CT system (table 2). For every 100 MBq increase in injected dose, bloodNaFmean increased by 0.35 kBq/mL. bloodNaFmean was significantly higher among subjects examined by the GE Discovery 690/710 than subjects examined by the GE Discovery VCT. Superior vena cava bloodNaFmean did not depend on variations in renal function or circulating time.

At all levels of the arterial tree, NaFmax was significantly affected by blood activity and PET/CT system. For every kBq/mL increase in bloodNaFmean, NaFmax increased by

0.92 to 0.97 kBq/mL for the aorta and by 0.86 kBq/mL for the coronary arteries. NaFmax was significantly higher among subjects examined by the GE Discovery 690/710 than subjects examined by the GE Discovery STE, VCT, or RX. In addition to blood activity and PET/CT system, descending thoracic aortac NaFmax was significantly affected by renal function (β = -.15, *P* = .014) and ascending aorta NaFmax was significantly affected by renal function (β = -.11, *P* = .020) and injected dose (β = .19, *P* = .008). NaFmax was not affected by variations in circulating time (table 3 and supplementary tables 4-6).

At all levels of the arterial tree, TBRmax/mean was significantly affected by blood activity and PET/CT system. For every kBq/mL increase in bloodNaFmean, TBRmax/mean decreased by 1.15 to 1.27 for the aorta and 1.63 for the coronary arteries. TBRmax/mean was significantly higher among subjects examined by the GE Discovery 690/710 than subjects examined by the GE Discovery STE, VCT, or RX. In addition to blood activity and PET/CT system, coronary TBRmax/mean was significantly affected by injected dose (β = .30, *P* = .020) and ascending aorta TBRmax/mean was significantly affected by injected dose (β = .33, *P* = .001) and renal function (β = -.12, *P* = .034). TBRmax/mean was not affected by variations in circulating time (table 3 and supplementary tables 4-6).

At all levels of the arterial tree, bsNaFmax was significantly affected by PET/CT system. bsNaFmax was significantly higher among subjects examined by the GE Discovery 690/710 than subjects examined by the GE Discovery STE, VCT, or RX. In addition to PET/CT system, descending thoracic aorta bsNaFmax was significantly affected by renal function (β = -.18, *P* = .016) and ascending aorta bsNaFmax was significantly affected by renal function (β = -.13, *P* = .019) and injected dose (β = .21, *P* = .006). bsNaFmax was not

affected by variations in blood activity or circulating time (table 3 and supplementary tables 4-6).

DISCUSSION

Prospective evaluation of 89 healthy subjects demonstrated that quantification of arterial ^{18}F-NaF uptake is significantly affected by blood ^{18}F-NaF activity, ^{18}F-NaF injected dose, and PET/CT system, but not renal function and ^{18}F-NaF circulating time. Therefore, blood activity, injected dose, and PET/CT system should be taken into account to generate unbiased estimates of arterial ^{18}F-NaF uptake.

To account for blood activity, it has been proposed to calculate the ratio between arterial wall ^{18}F-NaF uptake and blood ^{18}F-NaF activity, known as the target-to-background ratio (TBRmax/mean) (*3,5*). However, the TBRmax/mean has been criticized to be too dependent on variations in blood activity (*6*). Our study confirmed that TBRmax/mean is dependent on variations in blood activity. Therefore, quantifying arterial ^{18}F-NaF uptake as TBRmax/mean may result in biased estimates of arterial ^{18}F-NaF uptake. In contrast, our study demonstrated that bsNaFmax does not depend on blood activity. Therefore, we prefer to quantify arterial ^{18}F-NaF uptake as bsNaFmax over TBRmax/mean. It should be noted that our preference cannot be substantiated by our data alone. For that, autoradiographic and histologic analysis of arterial ^{18}F-NaF uptake is necessary.

In studies that investigate vascular calcification metabolism with ^{18}F-NaF PET/CT, blood activity is commonly estimated in the superior vena cava, inferior vena cava, or right atrium (*3-6,11-14*). However, it is not known if estimates of blood activity are comparable among vessel beds. Theoretically, blood activity should be similar in intensity throughout the body, especially after prolonged circulating times. Nonetheless, our study demonstrated

that estimates of blood activity differ significantly among vascular beds. In particular, recorded blood activity was higher in the right and left internal jugular vein compared with other vascular beds. Spillover activity from adjacent ^{18}F-NaF avid structures likely accounts for this observation. For example, we believe that spillover activity from the skeleton, including the sternum, clavicles, and cervical spine, increase blood ^{18}F-NaF activity estimates in the internal jugular veins. Similarly, we speculate that spillover activity from vascular calcifications may increase blood ^{18}F-NaF activity estimates in the aortic arch. Therefore, we advise to fix the location of blood ^{18}F-NaF activity estimation to the lumen of the superior vena cava, because this location is easy to identify and is largely devoid from spillover activity from adjacent ^{18}F-NaF avid structures. In addition, our study demonstrated that blood ^{18}F-NaF activity could be determined with higher inter- and intra-rater agreement at this location. The excellent rater agreement suggests that blood activity can be accurately estimated via placement of a single ROI as compared to multiple ROIs over several slices as propagated by some authors (*13*). In summary, standardized estimation of blood activity may reduce systematic errors and increase inter-study comparability.

In addition to blood activity, renal function affected quantification of arterial ^{18}F-NaF uptake. Renal function, expressed as MDRD-eGFR, negatively associated with NaFmax, TBRmax/mean, and bsNaFmax. Theoretically, impaired renal function prolongs tracer availability and may contribute to increased ^{18}F-NaF accumulation in vascular calcifications. However, our study demonstrated that blood ^{18}F-NaF activity did not depend on variations in MDRD-eGFR. Therefore, it seems unlikely that impaired renal function influences

quantification of arterial ^{18}F-NaF uptake by prolonging tracer availability. We believe that impaired renal function is a risk factor for the development of vascular calcification and consequently drives the degree of arterial ^{18}F-NaF uptake, instead of affecting its quantification. This view finds support in studies that demonstrated strong positive associations between impaired renal function and increased prevalence of vascular calcifications (*15-17*). We acknowledge, however, that the impact of renal function on quantification of arterial Na^{18}F uptake was studied in a healthy population. Therefore, it remains to be determined whether our findings can be replicated in a more diseased population, such as in patients with severe renal insufficiency.

In addition to blood activity and renal function, injected ^{18}F-NaF dose affected quantification of arterial ^{18}F-NaF uptake. The impact of injected ^{18}F-NaF dose is closely related to the distribution volume of ^{18}F-NaF. An increase in body size, and hence distribution volume, will decrease the uptake of ^{18}F-NaF in the target organ, such as the arterial wall. To overcome this problem, our study administered an ^{18}F-NaF dosage proportional to the subject's body weight. However, our regression models demonstrated that arterial ^{18}F-NaF uptake increased linearly to injected dose for some arteries, which suggests overcompensation for the negative impact of distribution volume on arterial ^{18}F-NaF uptake. Calculation of the standardized uptake value (SUV) may account for variations in injected dose and distribution volume of ^{18}F-NaF. The SUV is the decay-corrected activity concentration of ^{18}F-NaF (kBq/mL) adjusted for injected dose (MBq) and body surface area (cm2) or body weight (kg). However, the observed multicollinearity between injected dose, body weight, and body surface area prevented SUV from adequately correcting for

variations in injected dose and distribution volume of the tracer (supplementary figure 3). To overcome issues surrounding injected ^{18}F-NaF dose, we advise to administer a fixed ^{18}F-NaF dose in studies evaluating vascular calcification with ^{18}F-NaF PET/CT and to take the effect of distribution volume of the tracer separately into account.

In addition to blood activity, renal function, and injected dose, quantification of arterial ^{18}F-NaF uptake was affected by differences in PET/CT technology. Even though our imaging protocol adhered to international practice guidelines (*18*) and our PET/CT systems were calibrated to a phantom, subjects examined by the GE Discovery 690/710 had significantly higher arterial ^{18}F-NaF uptake than subjects examined on our older PET/CT systems (i.e. GE Discovery STE, VCT, or RX). Differences in imaging hard- and software likely account for this observation (supplementary table 1). It remains challenging to cross-calibrate PET/CT systems to overcome differences in imaging hard- and software, even if PET/CT systems are from the same vendor, as was the case in our study. Our study only considered PET/CT instrumentation from General Electric that differed in generation. Hence, we could not investigate the impact of PET/CT technology from different vendors on quantification of arterial ^{18}F-NaF. Therefore, we encourage additional research to determine whether differences in PET/CT technology are easier to overcome in systems that are similar in generation but differ in vendor. In addition, promising initiatives in ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT imaging, such as the EARL ^{18}F-FDG PET/CT accreditation program (*19*), may resolve issues surrounding differences in PET/CT technology and may contribute to improved inter-scan agreement in quantitative PET studies.

In contrast to blood activity, renal function, injected dose, and PET/CT system, ^{18}F-NaF-circulating time did not affect quantification of arterial ^{18}F-NaF uptake. In a previous study our group demonstrated that circulating time affects quantification of arterial ^{18}F-NaF uptake (*6*). In 38 subjects imaged at 45, 90 and 180 minutes after ^{18}F-NaF administration, we could demonstrate that the maximum SUV, a value related to NaFmax, and blood ^{18}F-NaF activity significantly decreases with time ($P < .001$ and $P < .001$, respectively), whereas the TBRmax/mean significantly increases with time ($P < .001$). The blood subtracted maximum SUV, a value related to bsNaFmax, was not affected by the circulating time ($P = 0.65$). In the present study, we fixed the circulating time of ^{18}F-NaF to approximately 90 minutes and demonstrated that quantification of arterial ^{18}F-NaF uptake was not affected by small variations in circulating time. Consequently, a fixed time between ^{18}F-NaF administration and PET/CT acquisition can adequately negate the impact of circulating time on quantification of arterial ^{18}F-NaF uptake.

Finally, our study demonstrated that quantification of arterial ^{18}F-NaF uptake and blood ^{18}F-NaF activity can be achieved with excellent inter- and intra-rater reliability and agreement (supplementary tables 2 and 3). This finding is consistent with previous reports (*5,6*).

An important strength of the present study is that we prospectively evaluated the effect of personal characteristics and technical factors on arterial ^{18}F-NaF uptake in a group of healthy subjects. By studying healthy subjects we limited bias from cardiovascular risk

factors on the generated results. However, studying a healthy population prevents extrapolating the results to a more diseased population. For example, only 6 % of subjects had impaired renal function (MDRD-eGFR < 60 mL/min/1.73 m^2). Therefore, we remain cautious in extrapolating our results to subjects with severe renal insufficiency. Second, although our study results were internally validated by bootstrap techniques, they lack external validation. To overcome this limitation, our study should preferably be repeated in a different population by different researchers. Third, ethical considerations prevented collection of arterial specimens for histologic examination. Therefore, we could not associate arterial ^{18}F-NaF uptake to histologic markers of vascular calcification. For similar reasons, we could not collect invasive blood samples to determine and compare blood activity estimates obtained by PET/CT imaging to the true blood ^{18}F-NaF activity. Finally, the notion that quantification of arterial ^{18}F-NaF uptake by TBRmax/mean is suboptimal compared to bsNaFmax cannot be substantiated by our data alone. Comparing arterial ^{18}F-NaF uptake to autoradiographic and histologic analysis of vascular calcification may be able to confirm the notion that bsNaFmax is a preferred quantifier of arterial ^{18}F-NaF uptake.

CONCLUSION

Prospective evaluation of 89 healthy subjects demonstrated that quantification of arterial ^{18}F-NaF uptake is affected by blood ^{18}F-NaF activity, injected dose, and PET/CT system. These factors should be accounted for in quantification methodologies to generate accurate estimates of arterial ^{18}F-NaF uptake.

DISCLOSURES

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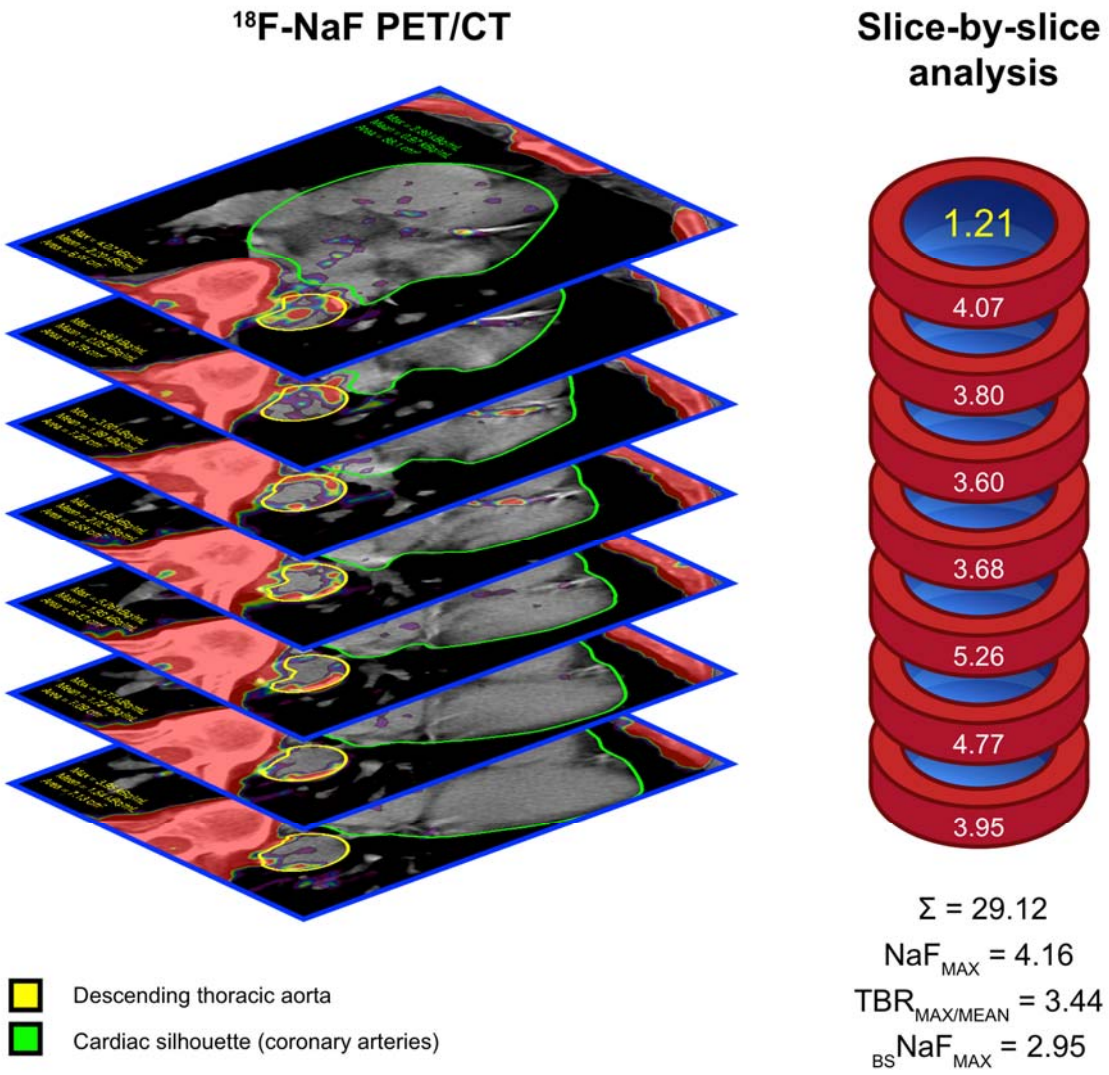

FIGURE 1 – Illustration demonstrating quantification of arterial sodium ^{18}F-fluoride (^{18}F-NaF) uptake. A region of interest (ROI) is drawn around the arterial wall (yellow ROI = descending thoracic aorta) or cardiac silhouette (green ROI) on every slice of the axially oriented ^{18}F-NaF PET/CT images. Per ROI, the maximum ^{18}F-NaF activity is determined. Values obtained per ROI are summed (Σ) and averaged (NaFmax) and subsequently divided or subtracted by the mean ^{18}F-NaF blood activity (bloodNaFmean). This provides the target-to-background ratio (TBRmax/mean) or the blood subtracted ^{18}F-NaF activity (bsNaFmax), respectively. Of note, bloodNaFmean was estimated in the superior vena cava (not shown).

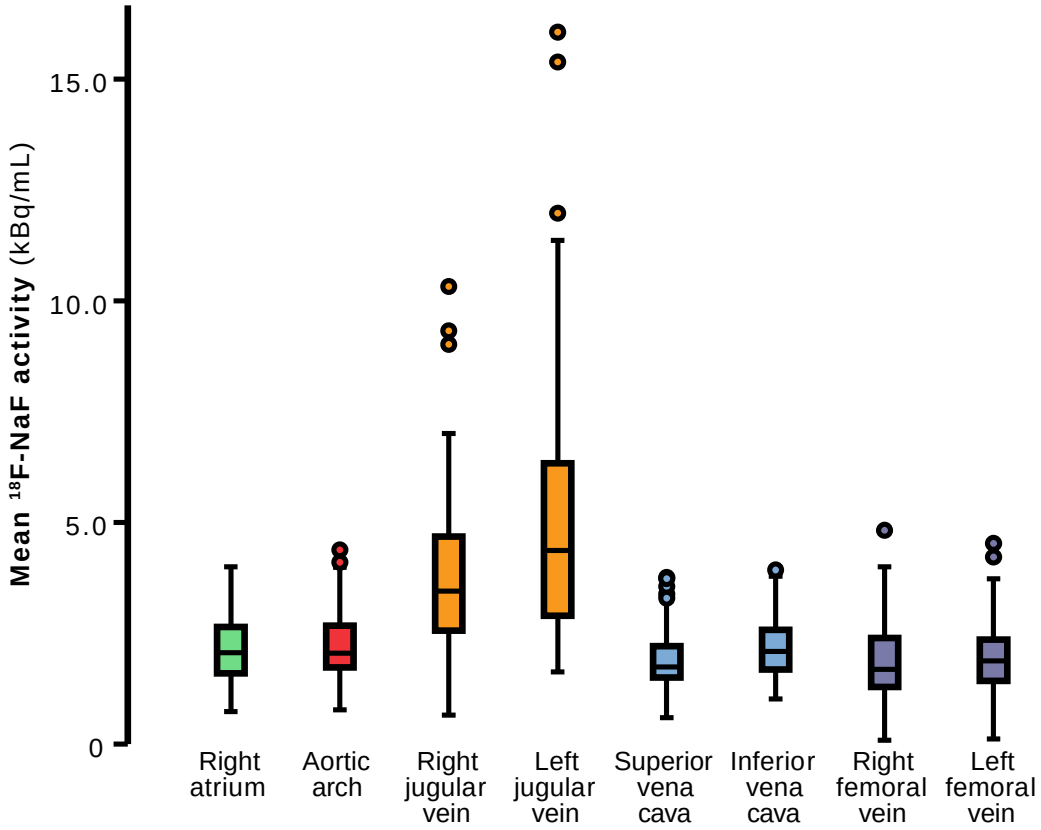

FIGURE 2 – Box plots of mean ^{18}F-NaF blood activity (bloodNaFmean) estimated in various vascular beds at 90 minutes after injection of ^{18}F-NaF. Blood activity in the right and left internal jugular vein was significantly higher (P < .001) compared with other vascular beds. Activity in the aortic arch was significantly higher compared with the superior vena cava (P < .001), inferior vena cava (P = .017), and right femoral vein (P = .001). bloodNaFmean was significantly lower in the superior vena cava than in the right atrium (P < .001) and inferior vena cava (P = 0.010). Filled circles represent outliers. Significance based on the repeated measures ANOVA with a Bonferroni correction.

TABLE 1 – **Subject demographics**

	Total (*N* = 89)	**Minimum**	**Maximum**
Age, years	44 ± 14	21	75
Male, *n* (%)	47 (53)		
Active smoking, *n* (%)	3 (3)		
Blood pressure, mmHg			
- Systolic	128 ± 17	98	201
- Diastolic	77 ± 10	57	107
Body weight, kg	80.2 ± 18.3	49.8	145.4
Body surface area, m^2	1.93 ± 0.24	1.54	2.67
Body mass index, kg/m^2	26.6 ± 4.4	17.6	42.5
Cholesterol, mmol/L			
- Total	4.9 ± 0.9	2.9	7.4
- LDL	3.1 ± 0.8	1.3	5.0
- HDL	1.4 ± 0.5	0.7	3.2
Triglycerides, mmol/L	1.0 ± 0.7	0.3	4.5
Plasma glucose, mmol/L	5.5 ± 0.5	4.4	6.7
HbA1c (mmol/mol)	33.9 ± 4.1	24.0	49.0
Creatinine, µmol/L	79.3 ± 13.1	52.0	118.0
MDRD-eGFR, mL/min/1.73 m^2	82.9 ± 13.2	55.0	113.0
Coronary calcium score			
- Agatston score, arbitrary units	0 [0 to 0]	0	1046
- Volume, mm^3	0 [0 to 0]	0	430
Injected dose, MBq	174 ± 39	109	348
Circulation time, minutes	92 ± 4	90	109
NaFmax, kBq/mL			
- Ascending aorta	3.32 ± 1.17	1.69	6.43
- Aortic arch	3.25 ± 1.07	1.36	7.16
- Descending aorta	3.22 ± 0.88	1.64	5.92
- Coronary arteries	3.75 ± 0.91	2.03	6.13
PET/CT system, *n* (%)			
- GE Discovery STE	22 (25)		
- GE Discovery VCT	19 (21)		
- GE Discovery RX	28 (31)		
- GE Discovery 690/710	20 (22)		

Values are mean ± standard deviation, *n* (%), or median [25 and 75 percentiles] for 89 subjects.

TABLE 2 – **Determinants of mean ^{18}F-NaF blood activity in the vena cava superior**

	Regression coefficient	**β**	**Adjusted R^2**	***P* value**
			.23	**< .001**
Intercept, kBq/mL	0.84 (0.42 to 1.49)			**.001**
Injected dose, 100 MBq	0.35 (0.03 to 0.55)	.32		**.011**
PET/CT system				
- GE Discovery STE	-0.14 (-0.36 to 0.13)	-.14		**.310**
- GE Discovery VCT	-0.39 (-0.61 to -0.16)	-.37		**< .001**
- GE Discovery RX	-0.01 (-0.23 to 0.19)	-.01		**.948**

The model eliminated renal function and circulating time. β = standardized regression coefficient. The 95 % confidence interval is presented in parentheses.

TABLE 3 – **Determinants of ^{18}F-NaF uptake in the ascending aorta**

	Regression coefficient	β	Adjusted R^2	*P* value
1. NaFmax, kBq/mL			.80	**< .001**
Intercept, kBq/mL	3.54 (2.59 to 4.23)			**< .001**
Blood activity, kBq/mL	0.94 (0.65 to 1.21)	.34		**< .001**
MDRD-eGFR, mL/min/1.73 m^2	-0.01 (-0.02 to -0.00)	-.11		**.020**
Injected dose, 100 MBq	0.57 (0.18 to 1.23)	.19		**.008**
PET/CT system				
- GE Discovery STE	-2.16 (-2.56 to -1.76)	-.80		**< .001**
- GE Discovery VCT	-2.14 (-2.55 to -1.74)	-.75		**< .001**
- GE Discovery RX	-1.99 (-2.37 to -1.61)	-.79		**< .001**
2. TBRmax/mean			.69	**< .001**
Intercept	4.89 (4.03 to 5.68)			**< .001**
Blood activity, kBq/mL	-1.27 (-1.75 to -0.90)	-.60		**< .001**
MDRD-eGFR, mL/min/1.73 m^2	-0.01 (-0.02 to -0.00)	-.12		**.034**
Injected dose, 100 MBq	0.77 (0.39 to 1.34)	.33		**.001**
PET/CT system				
- GE Discovery STE	-1.67 (-2.03 to -1.33)	-.80		**< .001**
- GE Discovery VCT	-1.54 (-1.89 to -1.20)	-.70		**< .001**
- GE Discovery RX	-1.55 (-1.86 to -1.24)	-.80		**< .001**
3. bsNaFmax, kBq/mL			.74	**< .001**
Intercept, kBq/mL	3.47 (2.63 to 4.10)			**< .001**
MDRD-eGFR, mL/min/1.73 m^2	-0.01 (-0.02 to -0.00)	-.13		**.019**
Injected dose, 100 MBq	0.55 (0.24 to 1.13)	.21		**.006**
PET/CT system				
- GE Discovery STE	-2.15 (-2.52 to -1.78)	-.91		**< .001**
- GE Discovery VCT	-2.12 (-2.49 to -1.78)	-.86		**< .001**
- GE Discovery RX	-1.99 (-2.33 to -1.62)	-.91		**< .001**

All models eliminated circulating time. In addition, blood activity was eliminated by model bsNaFmax.

β = standardized regression coefficient. The 95 % confidence interval is presented in parentheses.