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Associations of urinary isoprostanes with measures of subclinical atherosclerosis: The Multi-Ethnic Study of Atherosclerosis (MESA)

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ABSTRACT

Background: Urinary isoprostanes are markers of systemic oxidative stress, which is implicated in the pathogenesis of atherosclerotic cardiovascular disease (ASCVD). Coronary artery calcium (CAC), thoracic aortic calcium (TAC) and carotid plaque are measure subclinical atherosclerosis and prognosticate ASCVD risk. We examined the associations between urinary isoprostane levels and measures of plaque prevalence, burden, incidence and progression across three vascular beds in a cohort from the Multi-Ethnic Study of Atherosclerosis.

Methods: Urinary levels of 8-isoprostane and 2,3-dinor-8-F_2-isoprostane were measured in 1089 participants (mean ± SD 62 ± 8 years, 48% women) at baseline. Participants underwent computed tomography for CAC and TAC, and duplex ultrasound for carotid plaque. TAC and CAC were reassessed at 2.4 and 10 years, respectively. Regression models were adjusted for CVD risk factors.

Results: In adjusted models, there were no significant associations between isoprostane levels with CAC prevalence or progression. Highest versus lowest tertile of 8-isoprostane was associated with 28% lower prevalence of descending TAC at baseline [prevalence ratio (PR) 0.72 95% CI (0.56, 0.94)], while 1-SD higher 2,3-dinor-8-F_2-isoprostane was associated with 96% higher incident ascending TAC at follow-up [Relative Risk 1.96 (1.24, 3.09)]. Highest versus lowest tertile of isoprostane measures were associated with 22% higher prevalence of carotid plaque [(PR 1.22 (1.04, 1.45)] and 14% difference [3,26] in greater extent of carotid plaque at baseline.

Conclusions: Higher urinary isoprostanes were inconsistently associated with some measures of subclinical atherosclerosis by imaging. This suggests a limited role of urinary isoprostane levels as a prognostic marker for the development of ASCVD.

Trial registration: The MESA cohort design is registered at clinicaltrials.gov as follows: https://clinicaltrials.gov/ct2/show/NCT00005487.

Abbreviations: ASCVD, Atherosclerotic cardiovascular disease; ROS, reactive oxygen species; CVD, Cardiovascular disease; MESA, Multi-Ethnic Study of Atherosclerosis; iP$F_{2\alpha}$-III, 8-isoprostane; iP$F_{2\alpha}$-III-M, 2,3-dinor-8-F_2-isoprostane; CAC, coronary artery calcium; IMT, intima media thickness; CPS, Carotid plaque score; TAC, Thoracic aortic calcium; ATAC, Ascending thoracic aortic calcium; DTAC, Descending thoracic aortic calcium; eGFR, Estimated glomerular filtration rate; BMI, Body mass index; CT, Computed Tomography; HDL-C, high density lipoprotein cholesterol.

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Introduction

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of mortality and morbidity globally [1]. Given the systemic nature of ASCVD, multiple vascular beds can be affected. The development of subclinical atherosclerotic disease independently increases the risk of ASCVD events. For example, coronary artery calcium (CAC) scores and their progression have been well established to predict future CVD as well as both all cause and cardiovascular mortality [2–5]. Similarly, carotid plaque has been shown to predict cerebrovascular events such as stroke and transient ischemic attacks [6], even among individuals with CAC scores of zero [7]. Additional areas of extra-coronary calcium, such as calcification of the thoracic aorta and aortic arch, can also serve as predictors for the development of coronary artery disease and have a well described association with increased risk for stroke, cardiovascular mortality, and all-cause mortality [8–13]. While coronary plaque and extra-coronary atherosclerosis share many risk factors in common, they differ somewhat in terms of associated ASCVD risk and each provides incremental value for risk stratification [14–16].

Although the traditional risk factors for atherosclerotic disease are well defined, the role that systemic oxidative stress plays and our ability to quantify levels of oxidative stress are not as well established. Oxidative stress is defined by an imbalance between reactive oxygen species (ROS) and antioxidants, in which excess ROS can lead to endothelial injury and dysfunction [17]. Increased ROS production by macrophages reflects higher oxidative stress levels within plaque and is associated with plaque instability and progression [18]. Traditional cardiovascular disease (CVD) risk factors such as cigarette smoking, hypertension, diabetes, and hyperlipidemia are also implicated in ROS production [19].

These relationships between oxidative stress and the pathogenesis of ASCVD suggests that markers of systemic oxidative stress may have a role in predicting both coronary and extra-coronary plaque development and progression even after adjusting for traditional CVD risk factors. F2-isoprostanes, which are a prostaglandin like compound, are produced when ROS interact with arachidonic acid [20]. Their measurement therefore allows for quantification of the body's oxidative stress levels.

To help further elucidate the relationship between oxidative stress and atherosclerotic plaque progression across vascular beds, we examined the associations of the urinary isoprostanes 8-isoprostane ($iPF_{2\alpha}$-III) and its metabolite 2,3-dinor-8-F_2-isoprostane ($iPF_{2\alpha}$-III-M) with subclinical atherosclerotic disease within three different vascular beds in a cohort of men and post-menopausal women from the Multi-Ethnic Study of Atherosclerosis (MESA).

Design and methods

Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) enrolled 6,814 participants of White, Black, Hispanic and Chinese American race/ethnicity, ranging in age from 45 to 84 years old at the baseline exam in 2000–2002 [21]. The study centers include Baltimore County, Maryland; Chicago, Illinois; Forsyth County, North Carolina; New York, New York; Los Angeles County, California; and St Paul, Minnesota.

As part of an ancillary study [22,23], a subset (n = 1,289) of MESA participants free of CVD had urinary isoprostane levels measured from stored samples from the baseline exam and were included in this study population (Fig. 1). The sampling selection for this subcohort has been previously described [22–24]. It included men and post-menopausal women who had sex hormone levels measured at baseline and had cardiac magnetic resonance imaging at baseline and at 10-years as part of on-going work to understand sex differences in risk for heart failure with preserved ejection fraction [22,23]. For this analysis using the urinary isoprostane data, pre-menopausal women were excluded due to their small number (the sampling was intended to include only post-menopausal women by design) and those with reduced estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 because the urinary isoprostane values are indexed to urinary creatinine. Additionally, participants with insufficient urine for isoprostane measurement or who had missing covariates in our fully-adjusted model (model 3) were excluded. This left a final sample size of 1,089 for analysis for the computed tomography (CT) related measures (Fig. 1).

Measurement of Exposure variables

Questionnaires at baseline exam were used to determine information about age, sex, race/ethnicity, medical history, physical activity and smoking status. Smoking status was defined as either never smoking a cigarette, current cigarette use or past cigarette use, and pack year history was obtained as well for those with any history of cigarette use. Physical activity accounted for the frequency and intensity of the activity to calculate the estimated metabolic minutes per week by the study participants. Diabetes was defined as a fasting glucose greater or equal to 126 mg/dL or being on treatment for diabetes. Level of education was defined as either a high school level of education, some degree of college education or obtaining a college degree or higher. Body mass index (BMI) and blood pressure were measured by trained staff. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m2). Blood pressures were measured using a Dinamap model Pro 100 3 times in the seated position, and then the last two measurements were averaged and used in analysis. Hypertension was defined as a systolic blood pressure greater or equal to 140 mmHg, or a diastolic blood pressure greater or equal to

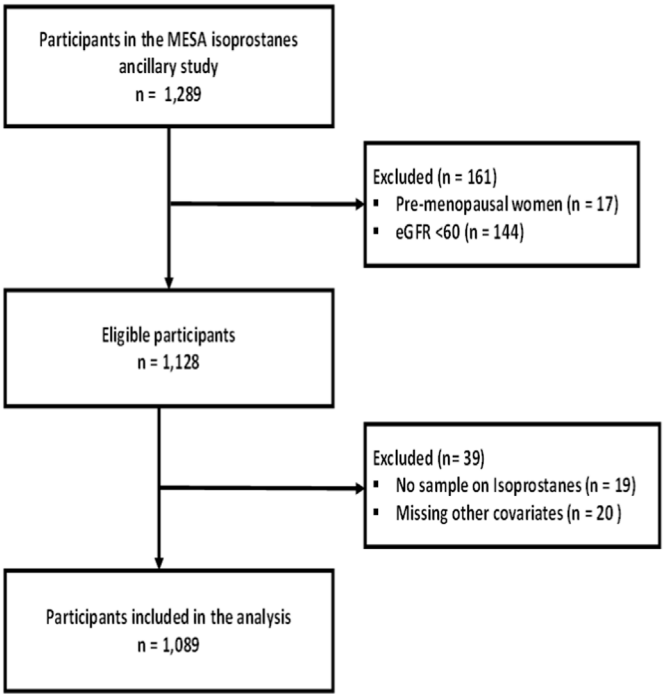

Fig. 1. Flowchart of study participants for CT analysis (CAC and TAC).

90 mmHg, or use of an antihypertensive medication. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) formula [25].

Total cholesterol and high density lipoprotein cholesterol (HDL-C) were measured from serum samples.

Urinary isoprostanes

Urinary isoprostane levels of 8-isoprostane ($iPF_{2\alpha}$-III) and its metabolite 2,3-dinor-8-isoprostane ($iPF_{2\alpha}$-III-M) were measured from urine samples at exam 1 (2000–2002). Samples underwent Mixed Anion Exchange-Solid Phase Extraction (MAX-SPE) for subsequent measurement of F2-isoprostanes by gas chromatography (GC-MS-NCI) as previously described [24,26]. Urinary isoprostanes were indexed to urinary creatinine measured using Jaffe rate method, to account for differences in urine concentration [27].

Measures of atherosclerosis

For this analysis, we examined the association of urinary isoprostanes with the presence and progression of plaque across three vascular beds: the coronary arteries (measured by CAC), the carotid arteries (measured by the carotid plaque score (CPS)), and the thoracic aorta (measured by thoracic aortic calcification (TAC)).

Coronary artery calcification

All participants received an electrocardiography (ECG)-gated cardiac CT scan at baseline exam 1 (2000–2002). During exam 5, which occurred about 10 years later (2010–2012), participants underwent repeat ECG-gated cardiac CT scan. CAC was measured by Agatston scoring. A CAC score of >0 at baseline indicated prevalent disease, and a CAC score >0 at follow-up among those with baseline scores of zero indicates incident disease.

Thoracic aortic calcification

TAC was also measured from the baseline cardiac CT scans (2000–2002). Although these scans were originally performed for assessment of CAC scoring, they were subsequently over-read for measurement of ascending (ATAC) and descending (DTAC) thoracic aortic calcification, measured by Agatston scoring [28–30]. The ascending thoracic aorta was defined as the portion of the aorta from the aortic root up until the takeoff of the brachiocephalic artery. The descending thoracic aorta was defined as the portion of the aorta from the left subclavian artery until the aortic hiatus of the diaphragm. A TAC score of 0 by Agatston scoring represented absence of disease at baseline, while baseline scores greater than 0 was used to determine baseline prevalence of disease. In addition to cardiac CT scan at baseline exam 1, participants also underwent repeat CT scan at an average of 2.4 years later during exams 2 or 3 timepoint (2002–2005). These CT scans were also over-read for TAC measurement. A TAC score >0 at follow-up among those with baseline scores of zero indicates incident disease.

Although CAC was measured again on a CT scan performed at exam 5 at 10-year follow-up, TAC has not yet been measured from those latter CT scans and only 2.4 year follow-up for TAC progression is available.

Carotid plaque scoring

A subset of participants underwent a carotid ultrasound at exam 1 (2000–2002). The overlap of participants with carotid ultrasound and urinary isoprostane measurement is different than for the CT scanning and is shown in Fig. 2 (n = 831). Carotid plaque was defined as a discrete, focal carotid wall thickening of ≥1.5 mm or focal thickening of at least 50% greater than the surrounding carotid intima media thickness [6,7]. The carotid plaque score (CPS) was then determined by the number of carotid plaques found in the internal, bifurcation and common segments of both carotid arteries. One point per plaque was allocated for the far and near walls of each of these carotid artery segments. The score's value ranged from 0 to 12.

Covariates

Covariates of interest were collected at the baseline exam through questionnaires, physical exam, and laboratory measures, as described previously [21]. Our study population was characterized with respect to baseline covariates, which included: age, sex, race/ethnicity, MESA center, BMI, education, smoking status, smoking pack-years, physical activity, systolic blood pressure, use of antihypertensive medications, history of diabetes, total cholesterol, HDL-C, use of lipid lowering medications, and eGFR.

Statistical analysis

We examined the outcomes of the three plaque measures (CAC, TAC, and CPS) separately. Poisson regression models with robust variance estimation were used to determine prevalence ratio (PR) for respective plaque scores >0 at baseline associated with isoprostane tertiles and per 1 standard deviation (SD) increment in isoprostane level. Relative risk (RR) ratios were derived from Poisson regression models with robust variance estimation for analysis of incident plaque score >0.

As has been done previously [29–31], we also examined progression in the plaque scores using multivariable-adjusted mixed-effects linear regression models to estimate cross-sectional and longitudinal associations between baseline isoprostane levels with baseline plaque extent and plaque progression. Given a high prevalence of plaque scores of 0, natural log of (plaque score +1) was used for these analyses. The beta-coefficients from the

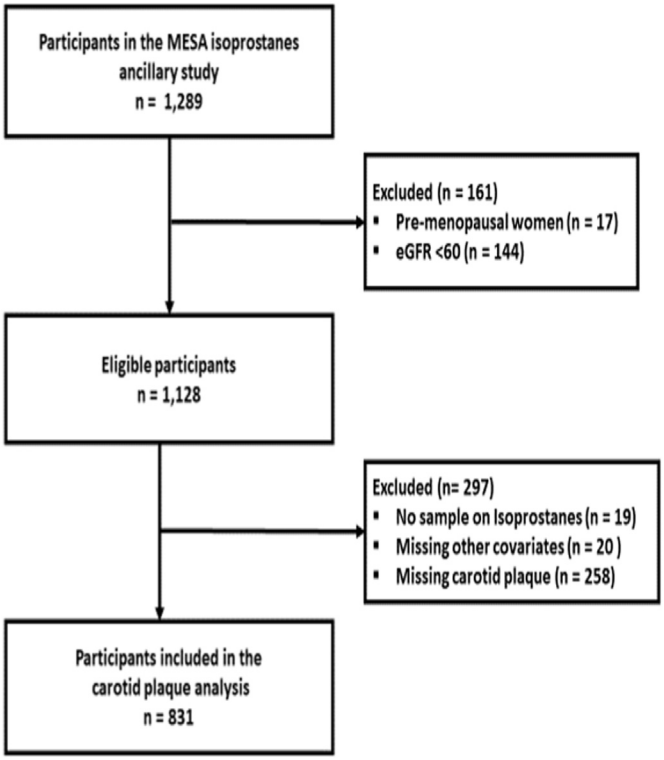

Fig. 2. Flowchart of study participants for carotid plaque analysis.

regression models were then exponentiated using the formula [Exp (β) −1] *100 to present the percent difference of plaque extent. These mixed effect analyses allowed baseline and longitudinal changes in plaque scores to be accounted for from all available time points.

For analyses for all three vascular beds, progressively adjusted models were used as follows:

Model 1: adjusts for age, sex, race/ethnicity, education, study center, and time between scans.

Model 2: adjusts for Model 1 + BMI, physical activity, smoking, and pack-years of smoking.

Model 3: adjusts for Model 2 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

Results

Study characteristics

The mean age (SD) of the 1,089 participants was 62 [8] years, of whom 48% were women, and 30% White, 17% Chinese-American, 30% Black, 24% Hispanic race/ethnicity. Full characteristics of study participants at baseline are listed in Table 1. The median (IQR) level of urinary 2, 3-dinor-8-F2-isoprostane was 3.1 (1.6, 5.9) ng/mL overall, and by tertiles was as follows: Tertile 1: 1.2 (0.7, 1.6) ng/ml, Tertile 2: 3.1 (2.5, 3.8) ng/ml, Tertile 3: 8.0 (5.9, 12.1) ng/ml. Participants with higher urinary isoprostane levels were more likely to be non-White race/ethnicity, have lower education, be current smokers, and have higher BMI.

Isoprostanes and TAC

Prevalent TAC was as follows: ATAC: 26/1089 (2.4%) and DTAC: 246/1089 (22.6%). Incident TAC was as follows: ATAC: 13/999 (1.3%) and DTAC: 90/796 (11.3%).

In cross-sectional analysis, there was no statistically significant association of isoprostanes with prevalent ATAC at baseline. However, there was a significant association between higher 8-isoprostane levels and 28% lower prevalence of DTAC at baseline in fully adjusted model for the highest tertile compared to the lowest (PR 0.72, 95% CI [0.56–0.94], Model 3, Table 2), which was contrary to expectation. Conversely, the metabolite, urinary 2,3-dinor-8-F2-isoprostane, was significantly associated with ATAC progression over a median of 2.4 years [RR 1.96 (1.24, 3.09)] per 1 SD increment (Model 3, Table 3). There was no significant association of isoprostane levels with TAC extent at baseline (Table 4) or progression over follow-up (Table 5) in the fully adjusted model (Model 3).

Isoprostanes and CAC

Prevalent CAC was found in 512/1089 (47.0%) individuals and incident CAC was seen in 217/448 (48.4%) individuals. There was a weak association between higher 8-isoprostane and lower prevalence of CAC at baseline, but this association did not remain when adjusted by additional covariates in the fully adjusted model 3 (PR 0.95, 95% CI [0.89–1.00], per 1 SD increment, Supplemental Table S1). There was no significant association of the metabolite, 2,3-dinor-8-F2-isoprostane, with CAC presence at baseline. There was no statistically significant association of either isoprostane with incident CAC at the 10-year follow-up (Supplemental Table S2). There was also no significant association of either isoprostane with CAC extent at baseline (Supplemental Table S3) or with CAC progression at follow-up (Supplemental Table S4).

Isoprostanes and carotid plaque

Prevalent carotid plaque was seen 402/831 (48.4%) individuals. For cross-sectional analysis of carotid plaque prevalence, there was a statistically significant association between the 3rd tertile of 8-isoprostane, compared to the 1st tertile, with baseline prevalence of carotid plaque in the fully adjusted model [PR 1.22, 95% CI 1.04, 1.45], model 3, Table 6). However, there was no association with the metabolite urinary 2,3-dinor-8-F2-isoprostane and carotid plaque presence (yes vs no). For baseline burden of carotid plaque, there was a statistically significant association between both isoprostane measures and baseline CPS (8-isoprostane of 14%, 95% CI [3, 26] and 2,3-dinor-8-F2-isoprostane of 13%, 95% CI [2, 25]), in the fully adjusted model 3, Table 7.

Discussion

In this multi-ethnic sample of individuals free of clinical CVD, elevated levels of urinary isoprostanes, a marker of oxidative stress, showed inconsistent associations with various measures of subclinical atherosclerotic plaque across three vascular beds. We found that higher isoprostane levels were associated with lower prevalence of DTAC, which is contradictory to our hypothesis, but did find higher isoprostane levels to be associated with higher incidence of ATAC and higher baseline burden of carotid plaque. These results raise many questions regarding the utility of isoprostanes as a biomarker for oxidative stress, and the role oxidative stress plays in the pathogenesis of ASCVD. It may be that single measures of urinary isoprostanes are insufficient to capture risk related to plaque presence and progression.

Oxidative stress profile

The data evaluating the role oxidative stress plays in the development of ASCVD are ever expanding, and the effect of traditional risk factors of ASCVD on levels of oxidative stress are already well established. Smoking, dyslipidemia and uncontrolled diabetes have all been shown to be associated with higher isoprostane levels, and therefore oxidative stress [32–35]. Previous studies have also shown increased urinary isoprotane levels being associated with increased mortality despite finding no association with CAC or carotid plaque measures [36].

However, in our study we were able to demonstrate significant, although occasionally weak, associations between isoprostanes and development of subclinical ASCVD in the form of greater ATAC incidence and progression but lower prevalence of DTAC, as well as greater carotid plaque prevalence and burden at baseline. The finding of lower baseline DTAC prevalence is not consistent with the overall association of higher isoprostane levels with greater ASCVD measurements. Taken together, these findings may suggest that urinary isoprostane levels may not be helpful for prognosticating ASCVD risk. Some individuals may have higher baseline levels of oxidative stress that are unrelated to currently understood risk factors. An underlying genetic predisposition for higher oxidative stress might explain these differences, but current studies are limited [37]. Perhaps repeated measures of isoprostane levels might integrate risk better, but we were unable to test that hypothesis. Further studies are needed to determine whether isoprostane levels may be useful in ASCVD risk stratification, especially in higher risk patients.

Given the role oxidative stress places in the etiology of ASCVD, reducing oxidative stress is a potential target for pharmacotherapy. Current therapies for atherosclerotic disease such as icosapent ethyl (eicosapentaenoic acid, EPA) reduce isoprostane levels, with the data being more inconsistent for statins [38,39]. Our study

Table 1
Baseline characteristics of study participants by 2, 3-dinor-8-F2-isoprostane tertiles.

N	Total	Tertile 1	Tertile 2	Tertile 3	p-value
	1,089	363	363	363	
8-Isoprostane, ng/ml	1.3 (0.7, 2.3)	0.6 (0.4, 1.1)	1.2 (0.8, 1.9)	2.5 (1.5, 3.7)	
Age, years	62 (8)	62 (8)	62 (9)	62 (8)	0.84
Male	561 (52%)	174 (48%)	193 (53%)	194 (53%)	0.25
Race/Ethnicity					
White	325 (30%)	126 (35%)	117 (32%)	82 (23%)	
Chinese-American	180 (17%)	90 (25%)	56 (15%)	34 (9%)	<0.001
Black	327 (30%)	79 (22%)	113 (31%)	135 (37%)	
Hispanic	257 (24%)	68 (19%)	77 (21%)	112 (31%)	
Education					
<High school	180 (17%)	60 (17%)	53 (15%)	67 (18%)	
High school, technical school, or associate degree	487 (45%)	138 (38%)	159 (44%)	190 (52%)	<0.001
College, graduate or professional school	422 (39%)	165 (45%)	151 (42%)	106 (29%)	
Smoking					
Never	606 (56%)	194 (53%)	217 (60%)	195 (54%)	<0.001
Former	364 (33%)	143 (39%)	112 (31%)	109 (30%)	
Current	119 (11%)	26 (7%)	34 (9%)	59 (16%)	
Pack-years of smoking>0	14 (6, 31)	13 (5, 28)	17 (6, 35)	14 (6, 32)	0.16
BMI, kg/m^2	28 (5)	27 (4)	28 (4)	29 (5)	<0.001
Physical activity, met-min/week	4290 (2130, 7800)	4230 (2175, 7245)	4140 (2070, 7590)	4470 (2130, 8670)	0.14
Systolic BP, mm Hg	125 (20)	126 (22)	125 (19)	125 (19)	0.37
Antihypertension medication	352 (32%)	114 (31%)	123 (34%)	115 (32%)	0.74
Total cholesterol, mg/dl	196 (37)	196 (35)	197 (39)	194 (36)	0.58
HDL cholesterol, mg/dl	50 (14)	51 (14)	51 (15)	49 (13)	>0.05
Lipid lowering medication	185 (17%)	60 (17%)	61 (17%)	64 (18%)	0.92
Diabetes	112 (10%)	33 (9%)	42 (12%)	37 (10%)	0.55
eGFR, mL/min/1.73 m^2	80 (13)	80 (12)	81 (13)	80 (13)	0.89

Data were presented as mean (SD) or number (percentage) or median (IQR).
To convert Total or HDL cholesterol from mg/dl to mmol/L, divide by 38.67.

Table 2
Prevalence ratio for TAC >0 at baseline.

	Model 1	Model 2	Model 3
ATAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	1.28 (0.55, 2.97)	1.32 (0.61, 2.89)	1.70 (0.74, 3.87)
3rd tertile	0.53 (0.18, 1.55)	0.54 (0.18, 1.58)	0.65 (0.22, 1.92)
Per 1SD log-transformed increase	0.80 (0.57, 1.12)	0.81 (0.59, 1.11)	0.84 (0.63, 1.13)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0.91 (0.40, 2.11)	1.17 (0.49, 2.78)	1.03 (0.41, 2.55)
3rd tertile	0.77 (0.31, 1.91)	0.71 (0.26, 1.92)	0.75 (0.29, 1.93)
Per 1SD log-transformed increase	0.79 (0.51, 1.23)	0.81 (0.51, 1.29)	0.84 (0.52, 1.34)
DTAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0.84 (0.66, 1.05)	0.83 (0.66, 1.04)	0.87 (0.69, 1.09)
3rd tertile	**0.72 (0.55, 0.93)**	**0.68 (0.52, 0.89)**	**0.72 (0.56, 0.94)**
Per 1SD log-transformed increase	0.93 (0.84, 1.03)	0.92 (0.83, 1.01)	0.95 (0.86, 1.04)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0.87 (0.68, 1.11)	0.85 (0.67, 1.08)	0.86 (0.68, 1.09)
3rd tertile	0.90 (0.70, 1.17)	0.89 (0.69, 1.16)	0.94 (0.73, 1.21)
Per 1SD log-transformed increase	0.96 (0.86, 1.07)	0.95 (0.85, 1.06)	0.97 (0.87, 1.09)

Abbreviations: ATAC, ascending thoracic aorta calcification; TAC, thoracic aorta calcification; DTAC, descending thoracic aorta calcification.
Prevalence ratios were derived from Poisson regression models with robust variance estimation.
Statistically significant results at $p < 0.05$ are in bold font.
Model 1: adjusts for age, sex, race/ethnicity, education and study center.
Model 2: adjusts for Model 1 + BMI, physical activity, smoking and pack-years of smoking.
Model 3: adjusts for Model 3 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

shows that isoprostanes and their metabolites have complex associations with markers of ASCVD. Currently, the clinical benefit of targeting oxidative stress in treating and preventing ASCVD remains unclear.

Lack of significance with CAC measures

Our findings showed a lack of statistical significance between urinary 8-isoprostane and its metabolite with any measurement of

Table 3
Relative risk of incident TAC >0 at Exam 2/3 (2002–2005) among participants with TAC = 0 at baseline.

	Model 1	Model 2	Model 3
ATAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	1.68 (0.38, 7.50)	1.73 (0.37, 8.05)	2.47 (0.46, 13.38)
3rd tertile	1.75 (0.40, 7.58)	1.21 (0.28, 5.17)	1.84 (0.46, 7.37)
Per 1SD log-transformed increase	1.21 (0.73, 1.99)	1.01 (0.66, 1.55)	1.11 (0.77, 1.61)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	1.90 (0.38, 9.57)	1.43 (0.26, 7.94)	1.32 (0.20, 8.80)
3rd tertile	3.56 (0.71, 17.80)	2.59 (0.54, 12.46)	3.67 (0.90, 14.94)
Per 1SD log-transformed increase	**1.94 (1.09, 3.47)**	1.64 (0.92, 2.92)	**1.96 (1.24, 3.09)**
DTAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0.86 (0.55, 1.36)	0.88 (0.56, 1.39)	0.94 (0.59, 1.51)
3rd tertile	0.78 (0.49, 1.23)	0.77 (0.49, 1.22)	0.85 (0.53, 1.37)
Per 1SD log-transformed increase	0.90 (0.76, 1.08)	0.89 (0.75, 1.06)	0.94 (0.79, 1.12)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0.80 (0.49, 1.30)	0.83 (0.52, 1.35)	0.89 (0.54, 1.45)
3rd tertile	1.07 (0.70, 1.64)	1.08 (0.71, 1.64)	1.22 (0.79, 1.86)
Per 1SD log-transformed increase	1.03 (0.86, 1.24)	1.03 (0.85, 1.24)	1.07 (0.88, 1.30)

Abbreviations: ATAC, ascending thoracic aorta calcification; TAC, thoracic aorta calcification; DTAC, descending thoracic aorta calcification.
Relative risks were derived from Poisson regression models with robust variance estimation.
Statistically significant results at $p < 0.05$ are in bold font.
Model 1: adjusts for age, sex, race/ethnicity, education, study center, and time between scans.
Model 2: adjusts for Model 1 + BMI, physical activity, smoking and pack-years of smoking. Model 3: adjusts for Model 3 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

Table 4
Cross-sectional association between baseline isoprostanes and log (TAC +1) at MESA Exam 1 (2000–2002) among men and post-menopausal women.

	Model 1	Model 2	Model 3
ATAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0 (−8, 10)	0 (−8, 9)	0 (−7, 9)
3rd tertile	−3 (−11, 5)	−3 (−11, 4)	−3 (−10, 5)
Per 1SD log-transformed increase	−2 (−6, 2)	−2 (−5, 2)	−1 (−5, 3)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	−4 (−11, 4)	−3 (−10, 4)	−3 (−10, 4)
3rd tertile	0 (−8, 10)	1 (−7, 11)	2 (−7, 12)
Per 1SD log-transformed increase	−1 (−4, 3)	0 (−4, 3)	0 (−4, 4)
DTAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	−11 (−34, 21)	−12 (−35, 20)	−8 (−31, 24)
3rd tertile	−25 (−44, 0)	**−27 (-45, -2)**	−19 (−40, 8)
Per 1SD log-transformed increase	−5 (−16, 8)	−5 (−16, 7)	−1 (−12, 12)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0 (−26, 34)	−2 (−27, 32)	0 (−25, 34)
3rd tertile	−5 (−30, 28)	−5 (−29, 29)	2 (−24, 37)
Per 1SD log-transformed increase	−2 (−14, 11)	−2 (−14, 10)	1 (−11, 14)

Abbreviations: ATAC, ascending thoracic aorta calcification; TAC, thoracic aorta calcification; DTAC, descending thoracic aorta calcification.
Results were presented as percent difference of TAC extent at baseline derived from mixed effect regression models and calculated from [Exp (β) −1] *100.
Statistically significant results at $p < 0.05$ are in bold font.
Model 1: adjusts for age, sex, race/ethnicity, education and study center.
Model 2: adjusts for Model 1 + BMI, physical activity, smoking and pack-years of smoking.
Model 3: adjusts for Model 3 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

CAC, except for a weak signal for lower prevalence in a limited adjusted model. However, CAC progression was positively but not significantly associated with the higher levels of urinary 2,3-dinor-8-F2-isoprostane.

Table 5
Longitudinal association between baseline isoprostanes and 2-year change in log (TAC +1) from MESA Exam 1 (2000–2002) to Exam 2/3 (2002–2005) among men and post-menopausal women.

	Model 1	Model 2	Model 3
ATAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	3 (−4, 11)	4 (−3, 11)	4 (−3, 11)
3rd tertile	3 (−3, 10)	3 (−3, 10)	4 (−3, 10)
Per 1SD log-transformed increase	1 (−2, 5)	1 (−2, 5)	2 (−2, 5)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	3 (−3, 11)	3 (−3, 11)	3 (−3, 11)
3rd tertile	3 (−3, 10)	3 (−3, 10)	3 (−3, 10)
Per 1SD log-transformed increase	2 (−1, 5)	2 (0, 5)	2 (0, 5)
DTAC			
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	−2 (−16, 13)	−2 (−16, 13)	−2 (−16, 13)
3rd tertile	−6 (−18, 9)	−6 (−18, 9)	−6 (−18, 9)
Per 1SD log-transformed increase	−3 (−9, 3)	−3 (−9, 3)	−3 (−9, 3)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	−8 (−19, 6)	−8 (−19, 6)	−8 (−19, 6)
3rd tertile	1 (−13, 17)	1 (−13, 17)	1 (−13, 17)
Per 1SD log-transformed increase	1 (−5, 7)	1 (−5, 8)	1 (−5, 8)

Abbreviations: ATAC, ascending thoracic aorta calcification; TAC, thoracic aorta calcification; DTAC, descending thoracic aorta calcification.
Results were presented as percent change of TAC progression at 2 years derived from mixed effect linear regression models and calculated from [Exp (β) −1] *100. Statistically significant results at $p < 0.05$ are in bold font.
Model 1: adjusts for age, sex, race/ethnicity, education and study center.
Model 2: adjusts for Model 1 + BMI, physical activity, smoking and pack-years of smoking.
Model 3: adjusts for Model 3 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

Carotid plaque measures

The highest tertiles of both isoprostane measures, compared to their respective lowest tertile, were significantly associated with greater baseline extent of carotid plaque, and 8-isoprostane was associated with greater likelihood of having prevalent plaque score >0. Perhaps isoprostanes might better capture carotid plaque risk by ultrasound, including softer plaque, compared to the CT-derived calcification measures which can only quantify calcified plaque.

Differences between ATAC and DTAC incidence

We found that the positive association between both urinary isoprostane levels with incidence of aortic calcifications existed only in the ascending thoracic aorta, but not the descending aorta. There are multiple possible explanations for this observed difference. First, the area of the descending aorta is larger than the ascending aorta, increasing the likelihood by which calcifications could have already existed at baseline. This is amplified by only needing a TAC measurement >0 by any amount to count towards incident TAC development. Second, the lower shear stress in the descending aorta from lower velocities promotes atherogenesis. These are likely reflected by the much higher baseline prevalence of DTAC versus ATAC (n = 306 vs n = 30, respectively) in our study. Previous studies have also shown higher rates of calcifications in the descending thoracic aorta [40]. It is possible that the higher prevalence of baseline DTAC in our study made it more difficult to elucidate associations with incidence, given the temporal nature of atherosclerotic disease [41,42].

Strengths and limitations

Strengths of our study include the use of a well-characterized cohort and the ability to examine the association of urinary isoprostanes with plaque and its progression across multiple different vascular beds. However, our study has a number of important limitations. Urinary isoprostane levels were measured only once, so we could not evaluate change in oxidative stress levels over time. Notably, urinary isoprostane levels were only measured in a subsample, which reduced our sample size. It is plausible that our study was not powered to detect statistically significant between isoprostanes and measures of plaque given small sample size and low event rates for progression. Based on the established prevalence of atherosclerosis in this cohort, we determined minimum detectable effect size with 80% power and 5% alpha error comparing the highest vs lowest tertile of isoprostanes and show this in Supplemental Table S5. As with all observational studies, our study design is limited by its non-randomized nature, which may not account for unmeasured factors that could be affecting ASCVD development. Additionally, multiple testing is an inherent limitation of our analyses, which increases the chance for erroneous inferences. Finally, for the TAC analysis, our study was also limited by an average observation period of 2.4 years for TAC measurements, whereas we had 10 years of follow-up for CAC progression. An association between isoprostanes and incidence of DTAC may have been observed in a larger population with a longer period of observation. Nevertheless, we think our findings are important

Table 6
Prevalence ratio for carotid plaque >0 at baseline.

	Model 1	Model 2	Model 3
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	1.00 (0.84, 1.19)	1.00 (0.84, 1.18)	1.03 (0.86, 1.23)
3rd tertile	**1.18 (1.00, 1.39)**	1.15 (0.97, 1.36)	**1.22 (1.04, 1.45)**
Per 1SD log-transformed increase	1.04 (0.97, 1.12)	1.03 (0.96, 1.10)	1.06 (0.99, 1.13)
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	0.97 (0.81, 1.15)	0.97 (0.82, 1.16)	0.97 (0.81, 1.15)
3rd tertile	1.09 (0.92, 1.29)	1.06 (0.89, 1.25)	1.11 (0.94, 1.32)
Per 1SD log-transformed increase	1.01 (0.94, 1.09)	1.00 (0.93, 1.08)	1.03 (0.95, 1.11)

Prevalence ratios were derived from Poisson regression models with robust variance estimation.
Statistically significant results at $p < 0.05$ are in bold font.
Model 1: adjusts for age, sex, race/ethnicity, education and study center.
Model 2: adjusts for Model 1 + BMI, physical activity, smoking, and pack-years of smoking.
Model 3: adjusts for Model 3 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

Table 7
Cross-sectional association between baseline log-transformed isoprostane and log (carotid plaque +1) at MESA Exam 1 (2000–2002) among men and post-menopausal women.

	Model 1	Model 2	Model 3
8-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	−1 (−11, 10)	−1 (−10, 10)	1 (−8, 12)
3rd tertile	10 (0, 23)	9 (−2, 21)	**14**)**3,26**)
Per 1SD log-transformed increase	3 (−1, 8)	3 (−2, 7)	**5 (0, 9)**
2,3-dinor-8-F_2-isoprostane			
1st tertile	Reference	Reference	Reference
2nd tertile	−1 (−11, 9)	−1 (−10, 10)	−1 (−10, 10)
3rd tertile	10 (−1, 23)	10 (−1, 22)	**13** (**2,25**)
Per 1SD log-transformed increase	2 (−2, 7)	2 (−2, 7)	4 (−1, 8)

Results were presented as percent difference of carotid plaque extent at baseline derived from mixed effect linear regression models and calculated from [Exp (β) −1] *100.
Statistically significant results at $p < 0.05$ are in bold font.
Model 1: adjusts for age, sex, race/ethnicity, education and study center.
Model 2: adjusts for Model 1 + BMI, physical activity, smoking, and pack-years of smoking.
Model 3: adjusts for Model 3 + systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL-C, use of lipid lowering medications, diabetes and eGFR.

exploratory work. Our overall inconsistent or null findings suggest that more studies are needed to fully elucidate whether urinary biomarkers of oxidative stress have utility in guiding therapy or prognostication in atherosclerotic disease.

Conclusions

In summary, increased levels of urinary isoprostanes were positively but non-significantly associated with CAC progression, while showing significant associations with baseline carotid plaque prevalence and burden, as well as both incidence of ATAC. These relationships existed even when adjusted for multiple traditional risk factors for ASCVD. However, there was also an association of lower prevalence of DTAC in those with higher isoprostane levels. Our ability to demonstrate consistent relationships may have been limited by sample size. Despite these inconsistent findings, the positive associations between isoprostanes and development of subclinical atherosclerotic disease in any vascular bed suggests they may play a role in development of atherosclerosis in other vascular beds, given the systemic nature of ASCVD. Further research into the role isoprostanes and oxidative stress play in the development of subclinical ASCVD is needed to determine whether they can be used as a biomarker of atherosclerotic risk or extent. Further studies are needed to help determine if isoprostane measurements may be more useful in specific, high risk patient populations, and whether patients with higher levels of isoprostanes would have benefit from therapies that target their higher levels of oxidative stress.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Unrelated to this work, Dr. Michos has served on advisory boards for Amgen, Amarin, AstraZeneca, Bayer, Boehringer Ingelheim, Esperion, Novartis, Novo Nordisk, and Pfizer. Dr Budoff has grants from the National Institute of Health and General Electric. Unrelated to this work, Dr. Hoogeveen has received research grants (to his institution) from Denka Seiken and is a consultant for Denka Seiken. No other author reports any conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2022.12.002.

References

[1] Tsao CW, Aday AW, Almarzooq ZI, et al. Heart disease and stroke statistics-2022 update: a report from the American heart association. Circulation 2022;145(8):e153–639.
[2] Budoff MJ, Young R, Burke G, et al. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). Eur Heart J 2018;39:2401–8.
[3] Budoff MJ, Young R, Lopez VA, et al. Progression of coronary calcium and incident coronary heart disease events: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol 2013;61:1231–9.
[4] Budoff MJ, Hokanson JE, Nasir K, et al. Progression of coronary artery calcium

predicts all-cause mortality. JACC Cardiovas Imag 2010;3:1229–36.
[5] Michos ED, Blaha MJ, Blumenthal RS. Use of the coronary artery calcium score in discussion of initiation of statin therapy in primary prevention. Mayo Clin Proc 2017;92:1831–41.
[6] Gepner AD, Young R, Delaney JA, et al. Comparison of carotid plaque score and coronary artery calcium score for predicting cardiovascular disease events: the multi-ethnic study of atherosclerosis. J Am Heart Assoc 2017;6:e005179.
[7] Mehta A, Rigdon J, Tattersall MC, et al. Association of carotid artery plaque with cardiovascular events and incident coronary artery calcium in individuals with absent coronary calcification: the MESA. Circulation Cardiovascular imaging 2021;14:e011701.
[8] Iribarren C, Sidney S, Sternfeld B, Browner WS. Calcification of the aortic arch: risk factors and association with coronary heart disease, stroke, and peripheral vascular disease. JAMA 2000;283:2810–5.
[9] Kianoush S, Al Rifai M, Cainzos-Achirica M, et al. Thoracic extra-coronary calcification for the prediction of stroke: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2017;267:61–7.
[10] Kalsch H, Mahabadi AA, Moebus S, et al. Association of progressive thoracic aortic calcification with future cardiovascular events and all-cause mortality: ability to improve risk prediction? Results of the Heinz Nixdorf Recall (HNR) study. European Heart J Cardiovas Imag 2019;20:709–17.
[11] Tison GH, Guo M, Blaha MJ, et al. Multisite extracoronary calcification indicates increased risk of coronary heart disease and all-cause mortality: the Multi-Ethnic Study of Atherosclerosis. J Cardiovas Computed Tomograph 2015;9:406–14.
[12] Obisesan OH, Osei AD, Berman D, et al. Thoracic aortic calcium for the prediction of stroke mortality (from the coronary artery calcium consortium). Am J Cardiol 2021;148:16–21.
[13] Santos RD, Rumberger JA, Budoff MJ, et al. Thoracic aorta calcification detected by electron beam tomography predicts all-cause mortality. Atherosclerosis 2010;209:131–5.
[14] Allison MA, Hsi S, Wassel CL, et al. Calcified atherosclerosis in different vascular beds and the risk of mortality. Arterioscler Thromb Vasc Biol 2012;32:140–6.
[15] Jacobs PC, Prokop M, van der Graaf Y, et al. Comparing coronary artery calcium and thoracic aorta calcium for prediction of all-cause mortality and cardiovascular events on low-dose non-gated computed tomography in a high-risk population of heavy smokers. Atherosclerosis 2010;209:455–62.
[16] Bos D, Leening MJ, Kavousi M, et al. Comparison of atherosclerotic calcification in major vessel beds on the risk of all-cause and cause-specific mortality: the rotterdam study. Circulat Cardiovas Imag 2015;8:e003843.
[17] Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. Curr Atherosclerosis Rep 2017;19:42.
[18] Channon KM. Oxidative stress and coronary plaque stability. Arterioscler Thromb Vasc Biol 2002;22:1751–2.
[19] Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol 2003;91:7A–11A.
[20] Montuschi P, Barnes PJ, Roberts LJ. 2nd. Isoprostanes: markers and mediators of oxidative stress. Faseb J 2004;18:1791–800.
[21] Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol 2002;156:871–81.
[22] Subramanya V, Zhao D, Ouyang P, et al. Cyclic guanosine monophosphate and 10-year change in left ventricular mass: the Multi-Ethnic Study of Atherosclerosis (MESA). Biomarkers 2021;26:309–17.
[23] Ying W, Zhao D, Ouyang P, et al. Associations between the cyclic guanosine monophosphate pathway and cardiovascular risk factors: MESA. J Am Heart Assoc 2019;8:e013149.
[24] Heravi AS, Michos ED, Zhao D, et al. Oxidative stress and menopausal status: the coronary artery risk development in young adults cohort study. J Wom Health 2022;31(7):1057–65.
[25] Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. Am J Kidney Dis 2010;55:622–7.
[26] Lee CY, Jenner AM, Halliwell B. Rapid preparation of human urine and plasma samples for analysis of F2-isoprostanes by gas chromatography-mass spectrometry. Biochem Biophys Res Commun 2004;320:696–702.
[27] Husdan H, Rapoport A. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. Clin Chem 1968;14:222–38.
[28] Ezeigwe A, Fashanu OE, Zhao D, et al. The novel inflammatory marker GlycA and the prevalence and progression of valvular and thoracic aortic calcification: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2019;282:91–9.
[29] Sharma A, Ogunmoroti O, Fashanu OE, et al. Associations of endogenous sex hormone levels with the prevalence and progression of valvular and thoracic aortic calcification in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2022;341:71–9.
[30] Sweeney T, Ogunmoroti O, Ndumele CE, et al. Associations of adipokine levels with the prevalence and extent of valvular and thoracic aortic calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2021;338:15–22.
[31] Subramanya V, Zhao D, Ouyang P, et al. Association of endogenous sex hormone levels with coronary artery calcium progression among postmenopausal women in the Multi-Ethnic Study of Atherosclerosis (MESA). J cardiovas Computed Tomography 2019;13:41–7.
[32] Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 1995;332:1198–203.
[33] Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. Circulation 1996;94:19–25.
[34] Davi G, Alessandrini P, Mezzetti A, et al. In vivo formation of 8-Epi-prostaglandin F2 alpha is increased in hypercholesterolemia. Arterioscler Thromb Vasc Biol 1997;17:3230–5.
[35] Davi G, Ciabattoni G, Consoli A, et al. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation 1999;99:224–9.
[36] Castro-Diehl C, Ehrbar R, Obas V, Oh A, Vasan RS, Xanthakis V. Biomarkers representing key aging-related biological pathways are associated with subclinical atherosclerosis and all-cause mortality: the Framingham Study. PLoS One 2021;16:e0251308.
[37] Forstermann U, Xia N, Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res 2017;120:713–35.
[38] Mas E, Woodman RJ, Burke V, et al. The omega-3 fatty acids EPA and DHA decrease plasma F(2)-isoprostanes: results from two placebo-controlled interventions. Free Radic Res 2010;44:983–90.
[39] Ky B, Burke A, Tsimikas S, et al. The influence of pravastatin and atorvastatin on markers of oxidative stress in hypercholesterolemic humans. J Am Coll Cardiol 2008;51:1653–62.
[40] Agmon Y, Khandheria BK, Meissner I, et al. Independent association of high blood pressure and aortic atherosclerosis: a population-based study. Circulation 2000;102:2087–93.
[41] Khoury Z, Gottlieb S, Stern S, Keren A. Frequency and distribution of atherosclerotic plaques in the thoracic aorta as determined by transesophageal echocardiography in patients with coronary artery disease. Am J Cardiol 1997;79:23–7.
[42] Gu X, He Y, Li Z, et al. Relation between the incidence, location, and extent of thoracic aortic atherosclerosis detected by transesophageal echocardiography and the extent of coronary artery disease by angiography. Am J Cardiol 2011;107:175–8.