Cardiovascular disease risk factors and oxidative stress among premenopausal women

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Abstract

Oxidative stress is one hypothesized mechanism linking anthropometric, behavioral, and medical risk factors with cardiovascular disease (CVD). We evaluated cross-sectional associations between CVD risk factors and biomarkers of oxidative stress, and investigated these biomarkers as predictors of incident diabetes and hypertension among premenopausal women. F_2-isoprostane (F_2-IsoP) and metabolite (15-F_{2t}-IsoP-M), reliable biomarkers of oxidative stress, were measured in urine samples collected at enrollment from 897 premenopausal women (ages 35-54) enrolled in the Sister Study cohort without a CVD history. Blood pressure, waist circumference, and body mass index (BMI) were measured at enrollment by trained study personnel. Diabetes and cigarette smoking were self-reported via enrollment questionnaires. Over a maximum follow-up of 11.5 years, participants self-reported incident diabetes and hypertension diagnoses on mailed questionnaires. In cross-sectional analyses, both F_2-IsoP and 15-F_{2t}-IsoP-M were positively associated with BMI, waist circumference, diastolic blood pressure, and current smoking. F_2-IsoP was elevated among those with diabetes, and 15-F_{2t}-IsoP-M increased with higher systolic blood pressure. Prospective analyses suggested an increased hypertension risk among those with elevated 15-F_{2t}-IsoP-M (highest vs. lowest quartile: hazard ratio=2.34; 95% CI: 1.20-4.56). Our results suggest that urinary F_2-IsoP and 15-F_{2t}-IsoP-M are positively associated with adiposity measures, blood pressure, and cigarette smoking. Further investigation is warranted to evaluate 15-F_{2t}-IsoP-M as a predictor of hypertension.
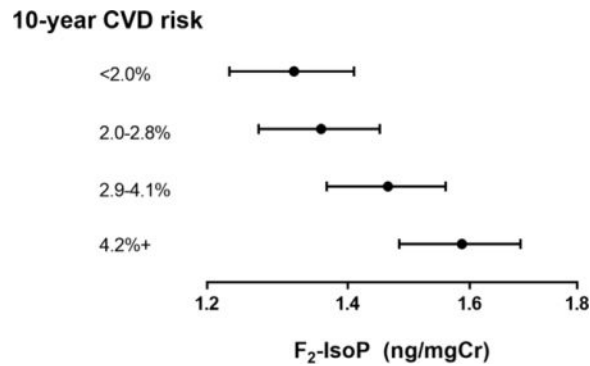
Graphical abstract

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Conflicts of interest

None

10-year CVD risk

Keywords

oxidative stress; F_2-isoprostanes; hypertension; cardiovascular disease; body mass index

Introduction

An overabundance of reactive oxygen species (ROS) relative to antioxidant defense, termed oxidative stress, may play a critical role in the pathogenesis of several chronic diseases, including cardiovascular diseases (CVD). ROS are hypothesized to drive the development and progression of atherosclerosis through the peroxidation of polyunsaturated fatty acids (PUFAs) in lipoproteins, producing a chronic inflammatory state that leads to plaque formation and rupture.[1] Thus markers of lipid peroxidation may be useful indicators of vascular disease risk.

Classical risk factors for atherosclerosis and subsequent occlusive events, such as obesity and cigarette smoking, have been associated with increased lipid peroxidation, a potential pathway through which these factors may influence vascular disease development.[1] F_2-isoprostanes (F_2-IsoPs), stable products of the peroxidation of arachidonic acid, are widely considered the current 'gold standard' measure of oxidative stress in vivo,[2] and elevations in both urinary and plasma concentrations of these biomarkers have been observed among current smokers and individuals with diabetes, hypertension, and obesity.[3–5] However, most previous investigations of relationships between F_2-IsoPs and these key CVD risk factors have been conducted in small, clinical cohorts, often comprised of patients with advanced disease. Furthermore, the few studies to date in healthy populations have relied exclusively on the measurement of plasma F_2-IsoPs or unmetabolized F_2-IsoPs in urine.[3–5] F_2-IsoPs measured in plasma may be subject to autoxidation during sample collection and storage,[6] while unmetabolized F_2-IsoPs in urine may reflect local F_2-IsoP production in the kidneys, rather than systemic oxidative stress.[7] Thus the predominant urinary F_2-isoprostane metabolite, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane (15-F_{2t}-IsoP-M), which is independent of local renal production, may be a better marker of systemic oxidative stress in vivo. To our knowledge, associations between 15-F_{2t}-IsoP-M and major CVD risk factors have not been comprehensively evaluated in a young, CVD-free population.

The objective of this study was to examine cross-sectional associations between risk factors for CVD and oxidative stress, as measured by urinary F_2-IsoP and 15-F_{2t}-IsoP-M, in a

cohort of premenopausal women without a history of cardiovascular conditions. Additionally, we evaluated whether urinary F_2-IsoP and 15-F_{2t}-IsoP-M concentrations were associated with incident diabetes and hypertension over a maximum follow-up of 11.5 years.

Materials and methods

Study population

Participants in these analyses were controls in a case-control study of oxidative stress and breast cancer risk nested within the prospective Sister Study cohort.[8] Between 2003 and 2009, over 50,000 women from the U.S. and Puerto Rico were recruited into the Sister Study through a national multi-media campaign and a network of breast cancer professionals and volunteers. Women were eligible to participate in the Sister Study if they were ages 35 to 74 years and free of breast cancer themselves at enrollment, but had a sister with a breast cancer diagnosis. All participants provided written informed consent. The study was approved by the Institutional Review Board of the National Institute of Environmental Health Sciences, the National Institutes of Health, and the Copernicus Group.

Sister Study participants eligible for inclusion in the control sample were ages 35 to 54 years, premenopausal, had at least one intact ovary, and had a urine sample collected at enrollment. Women were considered premenopausal if they self-reported at least one menstrual cycle within the 12 months prior to enrollment, or were aged 54 years and younger and their only reason for not experiencing menses was hysterectomy (without bilateral oophorectomy). A total of 922 women remained breast cancer free as of December 31, 2012 and were selected as control participants. For these analyses, we excluded women who reported a history of heart attack, angina, stroke, transient ischemic attack, or congestive heart failure (N=23) on enrollment questionnaires. We also excluded those classified as having type 1 diabetes, defined as a self-reported diabetes diagnosis before age 30, due to their small number (N=2). Thus final analyses include 897 women.

CVD risk factor assessment

During a home visit at Sister Study enrollment, trained study personnel used standardized protocols to measure blood pressure, height, weight, and waist circumference. Three sitting measurements, approximately 1–2 minutes apart, were taken for systolic and diastolic pressure. If both arms could be used, measurements were taken from alternating arms, starting with the left arm (Left→Right→Left). Otherwise, three readings were taken from the available arm. The average of the three readings was used in all analyses. Height and weight were measured without shoes, and waist circumference was measured using a cloth tape measure over skin or lightweight clothing. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Sociodemographic information and current cigarette smoking, physical activity, and use of medications for high blood pressure were assessed via questionnaire at Sister Study enrollment. Participants also self-reported any previous diagnosis of diabetes, age at diabetes diagnosis, and use of diabetes medication on enrollment questionnaires. We classified women as having type 2 diabetes if they reported a diabetes diagnosis at age 30 years or

older and/or were currently taking diabetes medication (self-reported diagnosis only: N=11; diabetes medication only: N=5; self-reported diagnosis and diabetes medication: N=15). According to the methods described by D'Agostino et al,[9, 10] we also calculated an absolute measure of 10-year general CVD risk. This risk score was developed in the Framingham Heart Study and incorporates information on non-laboratory-based risk factors, including gender, age, systolic blood pressure, hypertension treatment, smoking, diabetes, and BMI.

Incident diabetes and hypertension events were ascertained via detailed follow-up questionnaires, completed by participants every 2–3 years, and brief health update questionnaires completed annually. Women were asked to report a diagnosis of diabetes or hypertension, as well as the date of diagnosis.

Oxidative stress measurement

Sister Study participants provided first morning urine samples during the enrollment home visit. Urine samples were shipped frozen to the Eicosanoid Core Laboratory at Vanderbilt University Medical Center, where gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICI MS) was used to measure F_2-IsoP and 15-F_{2t}-IsoP-M. Detailed protocols for these methods have been published. [11–13] To account for urine diluteness, all values of F_2-IsoP and 15-F_{2t}-IsoP-M were adjusted for creatinine concentrations and are reported as ng/mg of creatinine (ng/mg Cr).

Statistical analysis

Values of F_2-IsoP and 15-F_{2t}-IsoP-M were log-transformed to approximate a normal distribution. Unadjusted and multivariable linear regression models, with log-transformed F_2-IsoP and 15-F_{2t}-IsoP-M as the dependent variable, were used to evaluate cross-sectional associations with continuous (age, systolic blood pressure, diastolic blood pressure, BMI, waist circumference, 10-year CVD risk score) and dichotomous (current smoking, prevalent diabetes,) variables. Due to evidence of collinearity between BMI and waist circumference, and between systolic and diastolic blood pressure, these variables were not entered together in the same linear regression models. We further considered adjustment for education level (less than Bachelor's degree, Bachelor's degree, higher than Bachelor's degree), race (white, non-white), and physical activity (total MET [metabolic equivalent] hours/week). Estimates were largely unchanged; therefore, we present results without adjustment for these additional variables. For associations between F_2-IsoP and 15-F_{2t}-IsoP-M and systolic and diastolic blood pressure measures, we performed sensitivity analyses excluding those who reported taking high blood pressure medication at enrollment (N=114).

Using general linear models, we calculated multivariable-adjusted geometric means of F_2-IsoP and 15-F_{2t}-IsoP-M within categories of all risk factor variables. BMI and waist circumference were categorized according to established guidelines,[14, 15] and systolic blood pressure, diastolic blood pressure, and 10-year CVD risk score were categorized using study-specific quartiles.

Cox proportional hazards regression models were used to estimate associations between F_2-IsoP and 15-F_{2t}-IsoP-M and incident diabetes and hypertension. Person-time at risk was

defined independently for each outcome, as the time between Sister Study enrollment and date of self-reported diagnosis or date of last contact, whichever came first. Participants who reported a diagnosis prior to Sister Study enrollment were excluded from analyses specific to that outcome (diabetes: N=46; hypertension: N=144). For incident hypertension analyses, we further excluded any others who reported high blood pressure medication use at enrollment (N=3), or had a systolic blood pressure 140 or a diastolic blood pressure 90, as measured at the enrollment home visit (N=2). F_2-IsoP and 15-F_{2t}-IsoP-M were categorized into quartiles for analyses of hypertension. Multivariable models for hypertension were adjusted for age, education, race, physical activity, BMI, and current smoking at enrollment. Tests for linear trend were conducted by including F_2-IsoP or 15-F_{2t}-IsoP-M in the model as a continuous variable. Due to the small number of incident diabetes events, we dichotomized F_2-IsoP and 15-F_{2t}-IsoP-M at the median for these analyses. In sensitivity analyses, we varied the cutpoint used for dichotomization (e.g. 25th or 75th percentile). Multivariable models for diabetes were adjusted for age, education, race, physical activity, and BMI at enrollment. The proportional hazards assumption was checked by visual assessment of log-log plots. All analyses were performed with Sister Study Data Release 5.0.1 using SAS 9.4 (SAS Institute, Cary, NC).

Results

Participant characteristics are shown in Table 1. The average age at enrollment was 47 years (SD= 4). The majority of participants were non-Hispanic white (87%) and non-smokers (91%). The mean BMI was 27 kg/m^2 (SD=7). Few participants had type 2 diabetes (3%), a diastolic blood pressure 90 (3%), or a systolic blood pressure 140 (2%) at enrollment.

In multivariable linear regression models, urinary F_2-IsoP and 15-F_{2t}-IsoP-M were positively associated with BMI (both p<0.001) and waist circumference (both p<0.001) (Table 2). Both F_2-IsoP and 15-F_{2t}-IsoP-M increased with increasing diastolic blood pressure (F_2-IsoP: p=0.031; 15-F_{2t}-IsoP-M: p<0.001), while only 15-F_{2t}-IsoP-M increased significantly with systolic blood pressure (p=0.004). Associations with blood pressure measures remained similar when 114 women taking high blood pressure medication at enrollment were excluded, for both F_2-IsoP (systolic: β=0.046, p=0.180; diastolic: β=0.063, p =0.007) and 15-F_{2t}-IsoP-M (systolic: β=0.087, p=0.002; diastolic: β=0.086, p<0.001). F_2-IsoP was elevated among current smokers (p=0.046) and those with type 2 diabetes (p=0.009). Patterns were similar for 15-F_{2t}-IsoP-M, though the association with type 2 diabetes appeared somewhat weaker (p=0.128). Age was inversely associated with 15-F_{2t}-IsoP-M (p=0.016), but was not strongly associated with F_2-IsoP. Both F_2-IsoP and 15-F_{2t}-IsoP-M increased with increasing 10-year CVD risk score (both p<0.001). Geometric means of F_2-IsoP and 15-F_{2t}-IsoP-M according to categorical variables are shown in Figure 1.

With adjustment for age and BMI at enrollment, neither F_2-IsoP nor 15-F_{2t}-IsoP-M concentrations above the median were strongly associated with incident diabetes (Table 3). Patterns remained similar when alternative cutpoints (e.g. 25th or 75th percentile) were used to dichotomize F_2-IsoP and 15-F_{2t}-IsoP-M (data not shown). F_2-IsoP was also not associated with risk of hypertension in the adjusted model. However, hypertension risk appeared to increase with 15-F_{2t}-IsoP-M (HR for highest vs lowest quartile: 2.34; 95% CI: 1.20, 4.56;

ptrend=0.060), though adjusted estimates were noticeably attenuated compared to unadjusted estimates.

Discussion

A number of behavioral, anthropometric, and medical factors may be associated with increases in lipid peroxidation, a potential mechanism through which these factors may contribute to CVD development and progression. In this study of premenopausal women without a CVD history, cross-sectional analyses suggested a positive association between F_2-IsoP and 15-F_{2t}-IsoP-M and BMI, waist circumference, blood pressure, diabetes, and smoking. These findings were reflected in the strong linear increase in F_2-IsoP and 15-F_{2t}-IsoP-M with increasing 10-year CVD risk score. In contrast, after adjustment for other risk factors, 15-F_{2t}-IsoP-M appeared to decrease with age. Additionally, our analyses suggested that elevated 15-F_{2t}-IsoP-M may be associated with an increased risk of developing hypertension.

Our findings for smoking and BMI are consistent with most previous investigations. The association between smoking and oxidative stress is well-established in the literature,[3, 16] reflecting the large number of oxidants present in cigarette smoke.[17] Several studies in diverse populations have also reported elevated concentrations of urinary or plasma F_2-isoprostanes among overweight (BMI 25.0-29.9 kg/m2) individuals and those with obesity (BMI 30.0 kg/m2).[3, 5, 18–22] Though fewer have evaluated associations with measures of central adiposity, such as waist circumference, our findings and those of others[4, 5, 18] suggest a strong linear relationship, similar to that for BMI. A number of mechanisms have been proposed to explain associations between adiposity and oxidative stress, including alterations in inflammatory markers and adipokines.[5]

In our cross-sectional analyses, both F_2-IsoP and 15-F_{2t}-IsoP-M were elevated among participants with diabetes, though the association with the metabolite was noticeably attenuated with adjustment for BMI and other risk factors. Other cross-sectional studies have also suggested higher F_2-IsoP concentrations among Type 2 diabetics.[3, 23] In a report from the Framingham Heart Study, which included men and women with and without a history of CVD, urinary F_2-IsoPs were positively associated with blood glucose and diabetes in age- and sex-adjusted models. The association with glucose remained significant in models adjusted for other CVD risk factors.[3] It remains unknown, however, whether diabetes is a cause or a consequence of oxidative stress,[24] and prospective studies to date have produced conflicting results. In the Insulin Resistance Atherosclerosis Study (IRAS), an inverse association was observed between several urinary F_2-IsoPs and diabetes risk,[25, 26] while a non-significant positive association was reported among Framingham Heart Study participants.[27] Given the small number of incident diabetes events in our sample, we were limited in our ability to evaluate F_2-IsoP and 15-F_{2t}-IsoP-M as predictors of diabetes risk. However, our results were not suggestive of an increased risk among those with elevated F_2-IsoP or 15-F_{2t}-IsoP-M.

The vasoconstricting and inflammatory properties of F_2-isoprostanes suggest their potential involvement in the development of hypertension. However, findings from previous studies of

relationships between oxidative stress and blood pressure in humans have been inconsistent. [3, 4, 28–35] Some have demonstrated positive associations between systolic blood pressure and F_2-isoprostanes[3, 4, 32] or higher F_2-isoprostane concentrations among hypertensives than among normotensives,[28, 33] though these associations are often attenuated by adjustment for BMI and other lifestyle and clinical factors.[3, 28, 32] Results of other cross-sectional and case-controls studies have suggested little association between F_2-isoprostanes and blood pressure,[30, 31, 34, 35] while a recent study among elderly Swedish men observed an inverse association between F_2-isoprostanes and a 24-hour measure of both systolic and diastolic blood pressure.[29]

In the current study, blood pressure measures were positively associated with F_2-isoprostanes in cross-sectional analyses, with particularly strong associations for $15\text{-}F_{2t}$-IsoP-M. These associations were apparent even at a relatively low distribution of blood pressure; the median values of systolic and diastolic blood pressure in our sample were only 110 and 71 mmHg, respectively, well below the clinical cutpoints for hypertension. Interestingly, our results also suggested that elevated $15\text{-}F_{2t}$-IsoP-M may be associated with incident hypertension. To our knowledge, this relationship has not been previously reported. In our sample, hypertension risk appeared to plateau between the 3rd and 4th quartiles of $15\text{-}F_{2t}$-IsoP-M. This may suggest a protective effect at the lowest $15\text{-}F_{2t}$-IsoP-M concentrations, but the absence of a linear dose-response relationship. Further research in larger and more diverse samples is warranted to prospectively characterize dose-response relationships between oxidative stress biomarkers and hypertension risk.

Strengths of the current study include the standardized assessment of anthropometric measures and blood pressure. The evaluation of $15\text{-}F_{2t}$-IsoP-M, in addition to F_2-IsoP, is also a notable strength. Our study also has limitations. For assessment of smoking status, diabetes, and medication use, we relied on self-reported information, which may be subject to inaccuracies. However, we were still able to detect associations between F_2-IsoP and $15\text{-}F_{2t}$-IsoP-M and several CVD risk factors, suggesting that these relationships are apparent even in young populations without a CVD history. Our definition of diabetes at enrollment included a small number of women who used diabetes medication but did not report a diabetes diagnosis (N=5). The definition of incident diabetes over follow-up did not include medication use. However, exclusion of women with medication only-defined diabetes at enrollment did not meaningfully alter the observed positive association with prevalent disease, providing confidence that this difference in definition is unlikely to have influenced our results for incident diabetes. We also relied on self-report of a physician diagnosis for our analyses of incident hypertension and diabetes, and were unable to account for potential differences in physician visits (or opportunities for hypertension/diabetes detection) according to enrollment characteristics. Finally, our sample was comprised entirely of premenopausal women, most of whom were non-Hispanic white. Thus our findings may not generalize to postmenopausal women, males, or individuals of other race/ethnicities.

Conclusions

Oxidative stress is one hypothesized mechanism linking several key risk factors with cardiovascular disease. Results of the current study suggest that urinary F_2-IsoP and $15\text{-}F_{2t}$-

IsoP-M, reliable biomarkers or oxidative stress, are positively associated with adiposity measures, blood pressure, and cigarette smoking. Our findings also suggest that elevated 15-F_{2t}-IsoP-M may be a predictor of incident hypertension. Further studies in larger, more diverse samples are warranted to confirm this association.

Acknowledgments

Abbreviations

CVD	cardiovascular disease
F_2-IsoP	F_2-isoprostane
15-F_{2t}-IsoP-M	F_2-isoprostane metabolite
BMI	body mass index
Cr	creatinine

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Highlights

- Oxidative stress may be a mechanistic link between key risk factors and CVD.

- F_2-isoprostanes (F2-IsoP) were positively associated with blood pressure measures.

- Higher F_2-IsoP was also associated with adiposity, diabetes, and current smoking.

- Elevated F_2-isoprostane metabolite in urine may predict incident hypertension.
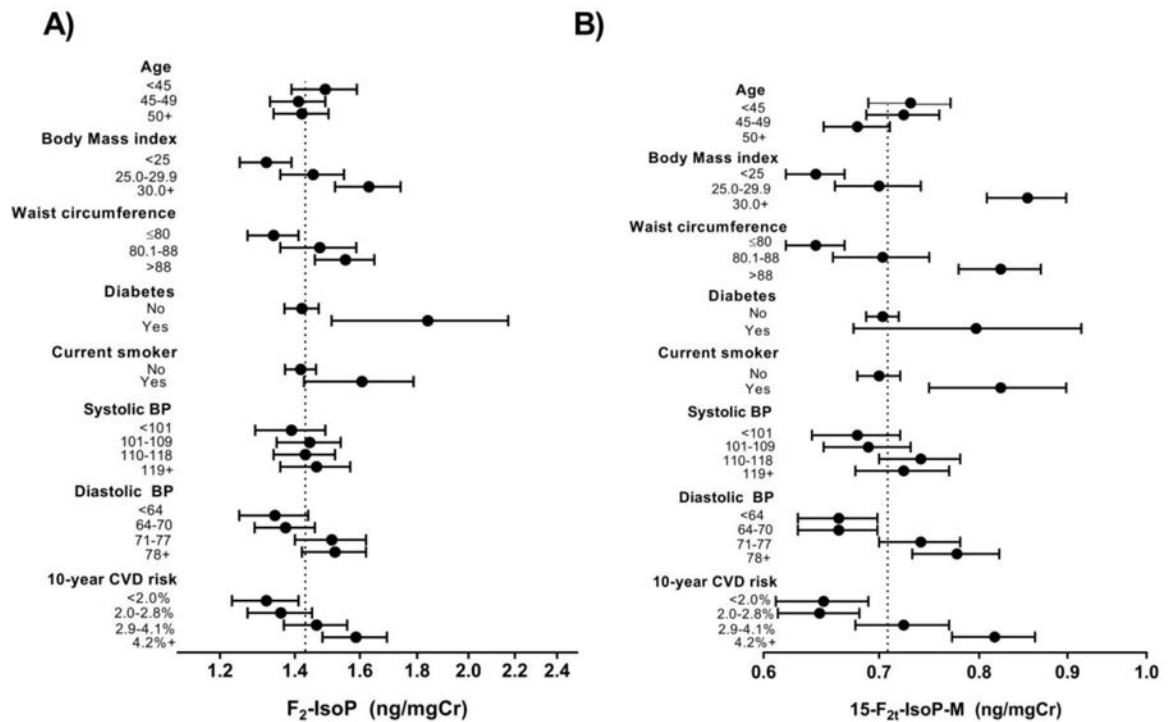
Figure 1.
Geometric mean (95% CI) values of A) F_2-IsoP and B) 15-F_{2t}-IsoP-M according to CVD risk factors among 897 premenopausal women. Means according to 10-year CVD risk are unadjusted. All other means adjusted for current smoking, prevalent diabetes and age. All means except for those according to waist circumference are additionally adjusted for body mass index. All means except for those according to diastolic blood pressure are additionally adjusted for systolic blood pressure.

Units: Body mass index, kg/m²; waist circumference, cm; systolic blood pressure, mmHg; diastolic blood pressure, mmHg

Table 1

Participant characteristics at enrollment (N=897)

	N	%
Age		
Mean, SD	47	4
<45	243	27
45-49	308	34
50+	346	39
Race/ethnicity		
Non-Hispanic white	784	87
Non-Hispanic black	57	6
Hispanic	34	4
Other	22	2
Education		
Less than Bachelor's degree	370	41
Bachelor's degree	284	32
Higher than Bachelor's degree	243	27
BMI (kg/m^2)		
Mean, SD	27	7
<25	418	47
25-29	244	27
30+	234	26
Waist		
Mean, SD	83	15
<=80	442	49
80.1-88	163	18
88+	290	32
Current smoking		
No	820	91
Yes	77	9
Total physical activity (MET-hrs/week), median (IQR)	44	(28, 66)
Type 2 Diabetes[*]		
No	866	97
Yes	31	3
Diastolic blood pressure (mmHg)[†]		
<64	207	23
64-70	255	28
71-77	198	22
78-89	212	24

	N	%
90	23	3
Systolic blood pressure (mmHg)[‡]		
<101	208	23
101-109	232	26
110-118	240	27
119-139	194	22
140	21	2
Currently taking high blood pressure medication		
No	783	87
Yes	114	13
10-year CVD risk (quartiles)		
<2.0%	201	22
2.0-2.8%	238	27
2.9-4.1%	225	25
4.2%	230	26

[*] Defined as a self-reported diabetes diagnosis at age 30 years or older and/or use of oral diabetes medication

[†] Categories defined by the 25th (64), 50th (71), and 75th (78) percentiles, and 90 mmHg, the value of diastolic blood pressure used to define hypertension

[‡] Categories defined by the 25th (101), 50th (110), and 75th (119) percentiles, and 140 mmHg, the value of systolic blood pressure used to define hypertension

Abbreviations: CVD, cardiovascular disease; BMI, body mass index; MET, metabolic equivalent; SD, standard deviation; IQR, interquartile range

Table 2

Regression coefficients for CVD risk factors in relation to change in log F_2-IsoP or 15-F_{2t}-IsoP-M (ng/mg Cr)

| | F$_2$-IsoP | | | | 15-F2t-IsoP-M | | | |
| | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[*] | |
	β	P	β	p	β	p	β	p
Systolic BP, per 20 mmHg	0.109	<0.001	0.019	0.524	0.169	<0.001	0.070	0.004
Diastolic BP, per 10 mmHg[†]	0.100	<0.001	0.046	0.031	0.138	<0.001	0.072	<0.001
BMI, per 5 kg/m^2	0.092	<0.001	0.079	<0.001	0.121	<0.001	0.101	<0.001
Waist circumference, per 5 cm[‡]	0.099	<0.001	0.080	<0.001	0.135	<0.001	0.109	<0.001
Current smoking	0.169	0.006	0.122	0.046	0.216	<0.001	0.165	<0.001
Type 2 diabetes	0.390	<0.001	0.249	0.009	0.326	<0.001	0.118	0.128
Age, per 5 years	0.015	0.439	0.026	0.182	0.020	0.234	0.038	0.016
10-year CVD risk	3.081	<0.001			4.205	<0.001		

*
Adjusted for systolic blood pressure, BMI, current smoking, prevalent diabetes, age

†
Adjusted for BMI, current smoking, prevalent diabetes, age

‡
Adjusted for systolic blood pressure, current smoking, prevalent diabetes, age Abbreviations: CVD, cardiovascular disease; F2-IsoP, F2-isoprostane; 15-F2t-IsoP-M, F2-isoprostane metabolite; BMI, body mass index; Cr, creatinine

Table 3

Hazard ratios (HR) and 95% confidence intervals (CI) for incident diabetes and hypertension

	N events	N person-years	Unadjusted HR (95% CI)	Adjusted HR (95% CI)[*]
Diabetes				
F_2-IsoP (ng/mg Cr)				
<1.38	14	3500.02	1	1
1.38+	23	3484.71	1.66 (0.85, 3.22)	1.14 (0.57, 2.29)
Continuous (per ng/mg Cr)			1.22 (0.95, 1.58)	0.98 (0.73, 1.30)
$15\text{-}F_{2t}$-IsoP-M (ng/mg Cr)				
<0.69	13	3550.93	1	1
0.69+	24	3433.81	1.91 (0.97, 3.75)	0.95 (0.45, 2.02)
Continuous (per ng/mg Cr)			2.21 (1.24, 3.94)	1.01 (0.48, 2.10)
Hypertension				
F_2-IsoP (ng/mg Cr)				
<1.00	20	1390.29	1	1
1.00-1.37	28	1443.45	1.37 (0.77, 2.44)	1.41 (0.79, 2.53)
1.38-1.94	29	1521.51	1.34 (0.76, 2.37)	1.19 (0.66, 2.14)
1.94+	37	1387.80	1.80 (1.04, 3.13)	1.31 (0.73, 2.35)
Continuous (per ng/mg Cr)			1.23 (1.06, 1.43)	1.06 (0.90, 1.24)
P_{trend}			0.006	0.482
$15\text{-}F_{2t}$-IsoP-M (ng/mg Cr)				
<0.53	14	1555.09	1	1
0.53-0.68	28	1510.49	2.07 (1.09, 3.93)	1.98 (1.04, 3.77)
0.69-0.93	33	1461.65	2.53 (1.36, 4.74)	2.34 (1.24, 4.43)
0.94+	39	1215.83	3.41 (1.85, 6.31)	2.34 (1.20, 4.56)
Continuous (per ng/mg Cr)			2.33 (1.59, 3.40)	1.51 (0.98, 2.33)
P_{trend}			<0.001	0.060

[*] Diabetes: adjusted for age, BMI, education, race, and physical activity; Hypertension: adjusted for age, BMI, education, race, physical activity, and current smoking

Abbreviations: F2-IsoP, F2-isoprostane; 15-F2t-IsoP-M, F2-isoprostane metabolite; Cr, creatinine