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Urinary F₂-isoprostanes and the risk of hypertension

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Abstract

Purpose: There is strong biological plausibility for a causal role of reactive oxygen species in vascular pathology but no direct epidemiological evidence linking elevated reactive oxygen species levels to hypertension development. We examined cross-sectional and prospective associations between oxidative status (urinary F₂-isoprostanes) and hypertension in the Insulin Resistance Atherosclerosis Study cohort ($n = 831$).

Methods: The cohort included non-Hispanic white, Hispanic, and non-Hispanic black individuals, with 252 (30%) having prevalent hypertension and 579 participants normotensive at baseline, 122 (21%) of whom developed hypertension during the 5-year follow-up. Four urinary F₂-isoprostane isomers were quantified in baseline specimens using LC/MS–MS and were summarized as a composite index. Examined outcomes included hypertension status (yes/no), systolic (SBP) and diastolic blood pressure (DBP), pulse pressure (PP), and mean arterial pressure (MAP).

Results: Prevalent and incident hypertension were associated with greater age, Black race, impaired glucose tolerance, and greater BMI. F₂-IsoP levels were lower among men and among non-Hispanic Blacks, were inversely associated with age, and were directly associated with BMI. No cross-sectional association was found between F₂-isoprostanes and hypertension status (OR = 0.93, 0.77–0.12). Among the continuous measures of blood pressure only PP was associated with F₂-isoprostanes at baseline (beta-coefficient = 0.99, 0.11–1.86). No prospective association was found between F₂-isoprostanes and incident hypertension: OR = 0.98, 0.77–1.25. No prospective associations were found for systolic blood pressure and diastolic blood pressure, and pulse pressure. Mean arterial pressure showed an inverse association (beta-coefficient = –0.16, –0.31 to –0.01).

Conclusions: Elevated F₂-isoprostane levels do not increase the risk of hypertension.

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No conflict of interest is reported.

Keywords

Oxidative stress; F₂-isoprostanes; Hypertension; Epidemiology; Cohort study

Introduction

The triumph of cholesterol-lowering interventions in reducing the risk of cardiovascular morbidity and mortality demonstrates the powerful synergy that is possible between the basic biological sciences and epidemiological studies [1]. A similar example of such synergy seemed to be the case with hypertension, a highly prevalent cardiovascular condition [2,3]. One of the most plausible causes of hypertension is oxidative stress, defined as a systemic overabundance of reactive oxygen species (ROS). The biological evidence strongly suggests that oxidative stress gives rise to endothelial dysfunction by reducing bioavailability of the key vascular regulator, nitric oxide, as well as increasing sodium and water retention and altering sympathetic outflow, all of which lead to an increase in blood pressure [4–13]. The strong biological plausibility of a causal relationship between oxidative stress and vascular disease has prompted large antioxidant supplementation trials in populations at high cardiovascular risk, but none of these have shown that antioxidants reduce blood pressure [1,14,15]. However, the absence of an antioxidant effect in supplementation trials does not directly answer the question of whether oxidative stress causes hypertension. It has been argued that the antioxidant trials did not use effective antioxidant regimens [16,17] and therefore, the results of the trials do not provide definitive evidence that oxidative stress is not a cause of hypertension. Indeed, none of the conducted antioxidant trials monitored oxidative stress measures in their participants. Without measuring systemic ROS levels, it is not possible to know whether a specific antioxidant regimen is effective in reducing oxidative stress.

The accepted method of measuring oxidative status is via non-enzymatically formed biomarkers of oxidative damage [18]. Extensive research has shown that F₂-isoprostanes (F₂-IsoPs), which are formed by the free radical-mediated peroxidation of arachidonic acid, serve as valid and reliable markers of oxidative status [19]. Currently, F₂-IsoPs present the only biomarkers that have been validated in both animal and human models of oxidative stress [20,21]. Thus, we reasoned that the best epidemiological evidence linking systemic ROS excess to hypertension risk should represent a prospective examination of the association between systemic F₂-IsoPs and hypertension incidence. To the best of our knowledge, only the cross-sectional results for the association between of F₂-IsoP levels and prevalent hypertension have been published, reporting both positive [22–25] and no associations [26,27]. In this report, we present the data of a prospective analysis within the well-characterized multiethnic cohort of the Insulin Resistance Atherosclerosis Study (IRAS) [28].

Methods

IRAS was a prospective epidemiological study designed to assess the relationships between insulin resistance, type 2 diabetes, cardiovascular disease, and other risk factors in a sample

of non-Hispanic white, Hispanic, and non-Hispanic black individuals. A total of 1625 men and women aged 40–69 at baseline were recruited between October 1992 and April 1994 from four clinical centers located in San Antonio, TX; San Luis Valley, CO; Oakland, CA; and Los Angeles, CA. In addition to obtaining racial/ethnic and geographic diversity, the IRAS study recruited participants who were metabolically diverse. The sampling methodology ensured adequate representation of groups with normal and impaired glucose tolerance (NGT and IGT) in addition to type 2 diabetes. The participants were followed for approximately five years. The IRAS included two physical examinations—at baseline and at 5-year follow-up. Baseline and follow-up examinations included an oral glucose tolerance test and assessment of demographic, lifestyle, and anthropometric characteristics. This analysis included participants free of type 2 diabetes at baseline ($n = 1125$); among them, 906 participants returned for the follow-up examination (with approximately 20% loss to follow-up), and 901 provided urine samples at baseline. The study was approved by the Institutional Review Committee of Wake Forest University School of Medicine and the subjects gave informed consent.

Each participant's blood pressure and hypertensive status was evaluated at baseline and in follow-up examinations. Using a standard mercury column sphygmomanometer, resting blood pressure was measured on three separate occasions during each examination. The average of the second and third measurements were used in determining blood pressure values and hypertensive status in this study. Mean arterial pressure (MAP) was calculated by adding two-thirds of the diastolic blood pressure (DBP) and one-third of the systolic blood pressure (SBP). Pulse pressure (PP) was calculated by subtracting diastolic blood pressure from systolic blood pressure. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, and/or a current regimen of antihypertensive medications.

During the baseline examination, morning spot urine samples were taken from all participants and stored at -70°C . F_2 -IsoPs were quantified using liquid chromatography/tandem mass spectrometry and adjusted for urinary creatinine concentration [29]. Out of 901 specimens, F_2 -IsoPs were successfully measured in 857 specimens, whereas F_2 -IsoPs could not be quantified in 44 specimens due to suppression of a mass spectrometry signal. A total of four F_2 -isoP isomers [iPF(2a)-III, 2,3-dinor-iPF(2a)-III, iPF(2a)-IV, 8,12-iso-iPF(2a)-IV] were evaluated and are referred to here as F_2 -IsoP1–4, respectively. To rank individuals based on all four measurements, we calculated F_2 -isoP index, which was previously used in the analysis of IRAS metabolomic data [30]. The F_2 -IsoP index variable was created by calculating the sum of four standardized isomers: $(X1_i - M1)/SD1 + (X2_i - M2)/SD2 + (X3_i - M3)/SD3 + (X4_i - M4)/SD4$, where X1–4 represent four F_2 -IsoP species, i —participant, M1–4 and SD1–4, mean and standard deviation values for the distributions of the respective F_2 -IsoP species.

Age, gender, race/ethnicity, and smoking status were self-reported in validated questionnaires. Normal glucose tolerance and impaired glucose tolerance status were determined by a 75-g oral glucose tolerance test. Body mass index (BMI), calculated as weight in kilograms divided by height in square meters for each participant, was used to represent overall adiposity.

Altogether, 831 nondiabetic participants were included in the current analysis, which excluded participants with missing values on any variable from either baseline or follow-up examinations ($n = 26$). The statistical analysis was performed using the SAS software package (version 9.3; SAS Institute, Cary, NC).

The examination of crude associations between hypertensive status and categorical study characteristics was carried out using χ^2 tests. The Wilcoxon rank-sum test was used to assess whether age, BMI, and F₂-IsoP index differed between those with and without prevalent and incident hypertension. To compare the F₂-IsoP index among the strata of categorical study characteristics, we used the Wilcoxon and Kruskal–Wallis tests. The Spearman correlation coefficient was used to examine the associations between the F₂-IsoP index and continuous variables.

Mixed-effects modeling was performed to assess adjusted cross-sectional and prospective associations between the F₂-IsoP index and the four continuous blood pressure variables: SBP, DBP, MAP, and PP. The models were adjusted for age (years), sex, race (black/non-black), and BMI (kg/m²). The three initial race/ethnicity categories were collapsed into two racial categories because the effect estimates between non-Hispanic whites and Hispanic whites were similar. Two random intercepts were included in the linear regression model to account for repeated blood pressure measurements on an individual and the clustering of individuals within clinic locations. In addition to a time invariant F₂-isoP index covariate, which describes the effect of baseline F₂-IsoPs on blood pressure measurements, the models included an interaction between time and F₂-IsoPs to assess the change in blood pressure over time with respect to its association with the baseline F₂-IsoP index. The following equation describes employed mixed models:

$$Y_{lij} = \beta_0 + \beta_1 t_{lij} + \beta_2 F_2 IsoP_{li} + \beta_3 t_{lij} * F_2 IsoP_{li} + X_{lij}^T \gamma + b_{l0} + b_{l(l)0} + b_{l(l)1} t_{lij} + \varepsilon_{lij}$$

where Y_{lij} is the j -th measurement of blood pressure (SBP or DBP or PP or MAP) for the i -th individual from the l -th clinic location, $\beta_0, \beta_1, \dots, \beta_3$ and γ are the fixed effects, $b_{l0}, b_{l(l)0}, b_{l(l)1}$ are random effects, ε_{lij} is random error, t_{lij} is the elapse time since baseline of the j -th visit of the i -th individual from the l -th clinic location, $F_2 IsoP_{li}$ is the baseline F₂-IsoP of the i -th individual from the l -th clinic location, and X represents a vector of covariates, including baseline age, sex, race, and BMI. $b_{l0} \sim \mathcal{N}(0, \sigma^2)$ is the random intercept accounting for the clustering effect of individuals within clinic locations, $b_{l(l)0}$ is the random intercept accounting for repeated blood pressure measurements on individuals, $b_{l(l)1}$ is the random slope. $b_{l(l)0}$ and $b_{l(l)1}$ are assumed to have a joint normal distribution with mean zero and an unspecified covariance matrix. The mean slope coefficient of baseline F₂-IsoP index is $(\beta_2 + \beta_3 t_{lij})$, which varies with time, and the effect of time on this slope coefficient is measured by β_3 . One-unit increase of baseline F₂-IsoP is expected to be associated with β_2 units change of baseline mean blood pressure value, and $(\beta_2 + \beta_3 t)$ units change of mean blood pressure after t years later. With the $t_{lij} * F_2 IsoP_{li}$ interaction, the mean slope coefficient of time is $(\beta_1 + \beta_3 F_2 IsoP_{li})$, which depends on the baseline F₂-IsoP; thus, β_3 also measures the effect of F₂-IsoP on the mean slope coefficient of time.

We performed hierarchical generalized linear modeling to assess adjusted cross-sectional and prospective associations between the F₂-IsoP index and hypertension status. The model was adjusted for baseline age (years), sex, race (black/non-black), and BMI (kg/m²). A random intercept was included to account for the clustering of individuals with respect to clinic location. The adjusted odds ratios were scaled by the standard deviation of the F₂-IsoP index predictor. The following equation describes our models with the binary outcome being hypertension status:

$$\text{logit}(P(Y_{li} = 1)) = \beta_0 + \beta_1 F2IsoP_{li} + X_{li}^T \gamma + b_{l0}$$

Y_{li} , assumed to have a Bernoulli distribution, indicates whether the i -th individual from the l -th clinic has hypertension, β_0 , β_1 , and γ are the fixed effects, $b_{l0} \sim \mathcal{N}(0, \sigma^2)$ is the random intercept accounting for the clustering effect of individuals within clinic locations.

Results

The ethnically and metabolically diverse study population included slightly more non-Hispanic whites (40%) than Hispanics (32%) and non-Hispanic blacks (28%). At baseline, one-third of the sample had IGT. Approximately one-fifth (19%) had a normal BMI (<25 kg per m²) at baseline, whereas nearly half (45%) were over-weight (25 ≤ BMI <30 kg per m²), and 36% were obese (BMI ≥ 30 kg per m²). The study included 252 participants with prevalent hypertension and 579 participants without hypertension at baseline (Table 1). Approximately 71% of the participants with prevalent hypertension at baseline reported taking antihypertension medications ($n = 180$). One-fifth of the individuals who were normotensive at baseline developed hypertension during the follow-up period (21% or 122/579). As expected, prevalent hypertension was associated with greater age, black race, and metabolic characteristics, including IGT status and greater BMI (Table 1). At baseline, normotensive participants showed a tendency to be current smokers more frequently than hypertensive individuals ($P = .07$). Incident hypertension showed a risk profile similar to that observed for the prevalent cases, including an association with BMI, although the associations with age and IGT status were attenuated. In addition, male sex was associated with incident hypertension (Table 1).

Urinary F₂-IsoP levels varied widely in this study population. We found 7-fold variation in individuals', as illustrated by the spread of values between the fifth and 95th percentiles in F₂-IsoP distributions: 0.06–0.62 ng per mg creatinine for F₂-IsoP, 1.32–9.66 for F₂-IsoP2, 2.26–14.91 for F₂-IsoP3, and 1.35–9.57 for F₂-IsoP4. The correlations between individual F₂-IsoP species varied, with the Spearman correlation coefficient being in the range of 0.6–0.7 ($P < .0001$). Each of the individual F₂-IsoP species strongly correlated with the F₂-IsoP index, with Spearman correlation coefficients between 0.76 and 0.84 ($P < .0001$), indicating that the index is a suitable summary measure for the four individual F₂-IsoP measurements. F₂-IsoP levels were consistently greater among females and lower among non-Hispanic blacks (Table 2). In addition to gender and race, the F₂-IsoP index showed inverse association with age and positive association with BMI (Table 2). Current smoking was associated with greater F₂-IsoP levels (Table 2).

We examined crude relationships of the blood pressure measures and hypertension with individual F₂-IsoP species and the F₂-IsoP index (Tables 1 and 2). Participants with incident hypertension tended to present with lower F₂-IsoP levels at baseline and, accordingly, the F₂-IsoP index showed a marginally significant crude association with incident hypertension (Table 1). The cross-sectional correlations of the continuous blood pressure measures (SBP, DBP, PP, and MAP) with F₂-IsoP species varied around the null, and no correlation were found with the F₂-IsoP index (Table 2). All further analyses were conducted using the F₂-IsoP index as a summary measure of the overall oxidative status.

We examined the relationships between F₂-IsoP levels and antihypertensive medication status. F₂-IsoP levels were elevated among participants with prevalent hypertension who were not taking medications ($n = 72$) as compared with those who reported being on medication ($n = 180$), with F₂-IsoP index of (-0.10) and (-0.34) , respectively ($P = .004$). However, F₂-IsoP index did not differ between prevalent hypertensive cases ($n = 72$) and normotensive participants ($n = 579$; $P = .25$).

The adjusted cross-sectional associations showed no association of F₂-IsoP index with hypertension status (OR = 0.93; 95% CI: 0.77, 1.12) and a tendency toward a positive association with the continuous blood pressure measures (Table 3). With respect to continuous variables, Table 3 presents beta-coefficients for 1-unit change in each variable with a unit of increase in F₂-IsoP index; for example, our model predicts that SBP increases by 1.08 with a unit of increase in F₂-IsoP index. Among the examined continuous variables, baseline PP was significantly and SBP marginally significantly associated with the F₂-IsoP index (Table 3). Additional adjustment for other potential confounders did not influence these associations.

Prospectively, incident hypertension was not associated with F₂-IsoP index (OR = 0.98; 95% CI: 0.77, 1.25), and the continuous measures of blood pressure showed a tendency for inverse associations with the baseline F₂-IsoP index (Table 3). In Table 3, the interaction between time and F₂-IsoP index shows the relationship between a change in a specific blood pressure variable during a year of follow-up associated with a unit of increase in F₂-IsoP index at baseline. Specifically, our model predicts that an individual with the baseline F₂-IsoP index 1 will experience less increase in SPB by -0.21 units annually (95% CI: -0.43 , 0.01) as compared with an individual with the baseline F₂-IsoP index of 0. Annual change in MAP was lower among individuals with elevated baseline F₂-IsoP index ($P = .04$); similar associations with changes in SBP and DBP were marginally significant ($P = .06$). We also found that baseline F₂-IsoP levels had no effect on annual changes in PP (Table 3).

Discussion

The major objective of the study was to examine the epidemiological evidence that elevated oxidative status increases the risk of hypertension. It is known that strong biological plausibility supported by epidemiological observations presents the best evidence-based platform for public health interventions. The success of cholesterol-lowering interventions in reducing cardiovascular morbidity and mortality represents one clear example. The story of antioxidant supplementation in the cardiovascular field reveals a perplexing paradox:

although there is solid biological evidence that oxidative stress plays an important role in vascular pathology [4–13], clinical trials have failed to demonstrate the expected beneficial effects of antioxidant supplementation [15]. However, these clinical trials did not directly assess the effect of antioxidants on oxidative status, nor did they monitor the changes in oxidative status while administering supplementation.

These methodological deficiencies of antioxidant clinical trials can be traced to the major gap that existed in the field at the time of the trials, namely, the lack of reliable measurements of oxidative status in human subjects. Over the past decade, however, systemic levels of F₂-IsoPs have emerged as a reliable measure of individual oxidative status, enabling epidemiological studies to examine the hypothesis of causal relationships between high oxidative status and hypertension [29]. We used state-of-the-art measurements of urinary F₂-IsoPs to characterize the oxidative status of participants in the multiethnic IRAS cohort, a cohort that is well characterized with respect to metabolic and cardiovascular risks. This nondiabetic cohort included participants with a wide spectrum of baseline BMI measures, normal and impaired glucose tolerance, normotensive and hypertensive status at baseline, and incident hypertension at the follow-up. The study population's diverse cardiovascular profile presents a unique opportunity to investigate both cross-sectional and prospective associations between oxidative status and hypertension.

In the IRAS study population, we found expected cross-sectional associations that are consistent with previously published data, serving as a “positive control” and suggesting generalizability of our results. These associations included greater prevalence and increased risk of hypertension among non-Hispanic blacks [31], associations of BMI with prevalent and incident hypertension [32,33], and greater levels of F₂-IsoPs among women and individuals with high BMI [34] (Tables 1 and 2). Importantly, our study population demonstrated wide variability of oxidative status as measured by urinary F₂-IsoPs, thus providing an opportunity to compare the occurrence of prevalent and incident hypertension among those with high versus low oxidative status. Our cross-sectional results found no positive association between the F₂-IsoP index and hypertension (Table 3). The significant cross-sectional association detected between PP and the F₂-IsoP index most likely signifies a connection between stiffness of the aorta and increased oxidative status, as PP increases with vessel stiffness (reduced compliance). However, the lack of a prospective association between the baseline F₂-IsoP index and PP at follow-up suggests that the observed cross-sectional association is not causal. Similarly, the increased oxidative status (or stress) among individuals with vascular pathology observed in this and some of the previous studies [22–25,35] can be interpreted as a consequence of these pathological processes. Furthermore, the lack of significant cross-sectional associations between the F₂-IsoP index and either prevalent hypertension or the other blood pressure measures suggest that the inconsistencies noted in previously published epidemiological results [26,27] can be interpreted as variation of spurious associations around the null.

Our prospective results, however, clearly demonstrate that normotensive individuals with high F₂-IsoP levels at baseline are not at an increased risk of developing hypertension or increasing in any blood pressure measurements (Table 3). To our knowledge, there are no published results with which we can compare our prospective findings. Taking into account

that the examined associations were adjusted for a minimal set of potential confounders and that the additional adjustment did not influence the results, we conclude that our results are not likely to have been driven by a confounding bias. In addition, the expected “positive control” associations point to the consistency of our data with previously published observations on the major risk factors for hypertension.

The findings from the present investigation may thus explain, in part, the results from antioxidant supplementation trials [1,14]. Inasmuch as we found no prospective relationship between F₂-IsoPs and blood pressure, it is not unexpected that long-term antioxidant supplementation has minimal, if any, effect on hypertension. Although there appears to be no relationship between free radical-derived oxidative stress and hypertension, it is possible that free radicals are not the most clinically important sources of oxidative stress. Alternatively, it is possible that a shift in redox balance through the nicotinamide nucleotide systems and thiol systems (protein thiols, glutathione, and associated disulfides), may be more important in development of hypertension [36]. Recent evidence has shown an association between nonfree radical oxidative stress and risk of death in patients with coronary artery disease [37]. Thus, if nonfree radical oxidative stress is more clinically relevant in the development of hypertension than free radicals, F₂-IsoPs are expected to show no association with this condition as reflecting free radical oxidative stress.

Conclusions

To date, no direct epidemiological evidence of a connection between free radical oxidative stress and hypertension has been published. Our study, with its careful prospective assessment of elevated oxidative status and hypertension, provides the best epidemiological data available on the lack of a causal relationship between them. This finding is in agreement with our previously published results, demonstrating that urinary F₂-IsoPs present a favorable factor that predicts lower risks of type 2 diabetes [38] and weight gain [39]. Together, our findings set the stage for a change in the existing paradigm that considers systemic F₂-IsoP levels as indicators of harmful oxidative stress. These findings inform future studies by suggesting more thorough inquiries into evidence-based mechanisms that adequately explain the relevance of experimental biology data on the role of oxidative stress in endothelial dysfunction and cardiovascular conditions.

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Table 1

Study characteristics and hypertension status

Characteristics	Cross-sectional (n = 831)		P*	Prospective (n = 579)		P*
	Normotensive at baseline	Hypertensive at baseline		Normotensive at follow-up	Hypertensive at follow-up	
N	579	252		457	122	
Age (years)	52 ± 14	58 ± 12	<.01	52 ± 13	54 ± 15	.14
Gender, n (%)			.9			.04
Male	246 (42.5)	107 (42.5)		184 (40.3)	62 (50.8)	
Female	333 (57.5)	145 (57.5)		273 (59.7)	60 (49.2)	
Ethnicity, n (%)			<.01			.04
Non-Hispanic white	248 (42.8)	85 (33.7)		198 (43.3)	50 (41.0)	
Non-Hispanic black	137 (23.7)	93 (36.9)		98 (21.4)	39 (32.0)	
Hispanic	194 (33.5)	74 (29.4)		161 (35.2)	33 (27.0)	
IGT status, n (%)			<.01			.09
Normal	420 (72.5)	141 (56.0)		339 (74.2)	81 (66.4)	
IGT	159 (27.5)	111 (44.0)		118 (25.8)	41 (33.6)	
Smoking status, n (%)			.07			.11
Current	91 (15.7)	25 (9.9)		75 (16.4)	16 (13.1)	
Past	218 (37.7)	107 (42.5)		162 (35.5)	56 (45.9)	
Never	270 (46.6)	120 (47.6)		220 (48.1)	50 (41.0)	
BMI (kg/m ²)	26.8 ± 5.2	28.9 ± 7.2	<.01	26.6 ± 4.9	27.3 ± 5.9	.02
Blood pressure						
SBP (mm Hg)	114 ± 17	135.5 ± 21.5	<.01	111.0 ± 14.5	123.5 ± 12.5	<.01
DBP (mm Hg)	75 ± 10.5	82 ± 13	<.01	74 ± 10	80.5 ± 9.5	<.01
PP	38 ± 12	51.5 ± 17.5	<.01	37.0 ± 10.5	43.3 ± 14.5	<.01
MAP	88.3 ± 11.2	99.3 ± 14	<.01	86.3 ± 11.0	94.2 ± 6.3	<.01
Blood pressure medication, n (%)			<.01			N/A
Yes	0 (0)	180 (71.4)		0 (0)	0 (0)	
No	579 (100)	72 (28.6)		457 (100)	122 (100)	
Oxidative status						
F ₂ -IsoP1, ng/mg CN	0.21 ± 0.17	0.19 ± 0.17	.05	0.21 ± 0.17	0.19 ± 0.15	.09

Characteristics	Cross-sectional (<i>n</i> = 831)		Prospective (<i>n</i> = 579)		<i>P</i> *
	Normotensive at baseline	Hypertensive at baseline	Normotensive at follow-up	Hypertensive at follow-up	
F ₂ -IsoP ₂ , ng/mg CN	3.69 ± 2.76	3.74 ± 2.74	3.87 ± 2.68	3.11 ± 2.51	.02
F ₂ -IsoP ₃ , ng/mg CN	3.48 ± 2.79	3.38 ± 2.80	3.47 ± 2.82	3.54 ± 2.77	.73
F ₂ -IsoP ₄ , ng/mg CN	5.57 ± 4.51	4.79 ± 3.48	5.68 ± 4.75	5.33 ± 3.15	.16
F ₂ -IsoP index	−0.18 ± 0.78	−0.28 ± 0.85	−0.13 ± 0.80	−0.25 ± 0.78	.08

IGT = impaired glucose tolerance; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; MAP = mean arterial pressure.

* Categorical variables were reported as N, (%) and assessed using Chi-square tests. Continuous variables were reported as median ± IQR and assessed using the Kruskal–Wallis tests.

Table 2

Associations between F₂-IsoPs (ng/mg creatinine) and baseline study characteristics

	F ₂ -IsoP1 [iPF(2α)-III]	F ₂ -IsoP2 [2,3-dimor-iPF(2α)-III]	F ₂ -IsoP3 [8,12-iso-iPF(2α)-IV]	F ₂ -IsoP4 [iPF(2α)-IV]	F ₂ -IsoP Index
Categorical baseline characteristics; median (interquartile range)					
Gender					
Male	0.16 (0.12)	2.95 (1.84)	3.20 (2.21)	4.22 (2.78)	-0.43 (0.55)
Female	0.24 (0.19)	4.46 (3.16)	3.59 (3.31)	6.38 (4.77)	0.00 (0.98)
P-value*	<.01	<.01	<.01	<.01	<.01
Ethnicity					
Non-Hispanic white	0.20 (0.15)	3.56 (2.70)	3.47 (2.31)	5.35 (3.96)	-0.25 (0.75)
Non-Hispanic black	0.16 (0.13)	3.23 (2.30)	2.90 (2.13)	4.31 (2.86)	-0.44 (0.60)
Hispanic	0.25 (0.21)	4.41 (3.03)	4.03 (3.60)	6.88 (4.85)	0.05 (0.95)
P-value*	<.01	<.01	<.01	<.01	<.01
IGT status					
Normal	0.20 (0.17)	3.61 (2.59)	3.36 (2.68)	5.39 (3.96)	-0.23 (0.78)
IGT	0.20 (0.16)	4.01 (3.12)	3.54 (2.98)	5.26 (4.74)	-0.23 (0.78)
P-value*	.52	.03	.27	.98	.34
Smoking status					
Current	0.26 (0.18)	4.10 (2.72)	3.59 (3.17)	5.82 (4.21)	-0.01 (0.91)
Past	0.18 (0.14)	3.51 (2.64)	3.28 (2.55)	5.03 (3.89)	-0.30 (0.70)
Never	0.19 (0.17)	3.69 (2.83)	3.52 (2.93)	5.43 (4.61)	-0.20 (0.83)
P-value*	<.01	.05	.13	<.01	<.01
Continuous baseline characteristics; Spearman correlation coefficients (P-value)					
Age (years)	-0.01 (.84)	-0.03 (.40)	-0.2 (<.01)	-0.03 (.39)	-0.07 (.05)
BMI (kg/m ²)	-0.001 (.98)	0.18 (<.01)	0.1 (<.01)	0.04 (.29)	0.09 (.01)
SBP (mm Hg)	-0.03 (.46)	0.05 (.14)	-0.03 (.45)	-0.07 (.05)	-0.01 (.70)
DBP (mm Hg)	-0.08 (.02)	-0.04 (.25)	-0.002 (.96)	-0.09 (.01)	-0.06 (.09)
PP	0.01 (.69)	0.09 (.01)	-0.05 (.16)	-0.03 (.38)	-0.01 (.71)
MAP	-0.06 (.08)	0.01 (.84)	-0.01 (.72)	-0.09 (.01)	-0.04 (.25)

* Wilcoxon and Kruskal-Wallis tests.

Table 3Cross-sectional and prospective associations between urinary F₂-IsoPs and blood pressure characteristics[†]

Outcome [†]	Associations between baseline F ₂ -isoP index and blood pressure [‡]	<i>P</i>	Associations between baseline F ₂ -isoP index and annual change in blood pressure [§]	<i>P</i>
	Beta-coefficient for F ₂ -isoP Index (95% CI)		Beta-coefficient for time *F ₂ -isoP Index (95% CI)	
N	579		579	
SBP	1.08 (−0.07 to 2.23)	.07	−0.21 (−0.43 to 0.01)	.06
DBP	0.29 (−0.47 to 1.04)	.45	−0.13 (−0.26 to 0.01)	.06
PP	0.99 (0.11 to 1.86)	.03	−0.08 (−0.24 to 0.08)	.35
MAP	0.47 (−0.33 to 1.28)	.25	−0.16 (−0.31 to −0.01)	.04
Association between baseline F₂-IsoP index and Hypertension, OR (95% CI)				
	Cross-sectional		Prospective	
	(252/579, cases/noncases)		(122/457, cases/noncases)	
N	831		579	
Hypertension	0.93 (0.77 to 1.12)	.45	0.98 (0.77 to 1.25)	.87

[†] All models adjusted for age, sex, race, and BMI; a random effect intercept is included to capture clustering effect of clinic.[‡] Models include baseline blood pressure measurements among normotensive participants.[§] Models include blood pressure measurements at baseline and follow-up and a random intercept effect to account for repeated measurements on an individual level.^{||} Odds ratios scaled by F₂-isoP index respective standard deviations.