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OXIDATIVE STATUS AND HYPERTENSION:
AN EXAMINATION OF THE PROSPECTIVE ASSOCIATION BETWEEN URINARY
F₂-ISOPROSTANES AND HYPERTENSION.

By
Charles David Melton

A Thesis Submitted to
The Graduate Faculty of Georgia State University
In Partial Fulfillment of the Requirements for the Degree

MASTER OF PUBLIC HEALTH

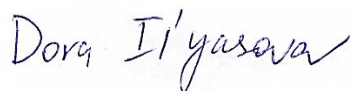
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AN EXAMINATION OF THE PROSPECTIVE ASSOCIATION BETWEEN URINARY
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Abstract

Background: Hypertension is a pathological increase in blood pressure that affects nearly 30% of the U.S. population and is a primary modifiable risk factor for cardiovascular disease. Despite advancements in prevention and treatment, hypertension is still one of the most common conditions around the world, and for a majority of cases the causal mechanisms remain to be fully elucidated. A growing body of literature suggests that oxidative stress status may play an etiological role in many chronic conditions, including hypertension. Specifically, a systemic overabundance of reactive oxygen species may give rise to endothelial dysfunction, increased sodium and H₂O retention, and alterations in sympathetic outflow, leading to an increase in blood pressure.

Purpose: The main objective of this study is to investigate the prospective association between F₂-isoprostanes, a validated biomarker of oxidative status, and development of hypertension in a large, multi-centered, multi-ethnic cohort of adults aged 40-69 at baseline.

Methods: This is a secondary data analysis that utilized previously collected data from the Insulin Resistance Atherosclerosis Study. 844 participants were included in the analysis. Briefly, four urinary F₂-isoprostane isomers (F₂-IsoP1, F₂-IsoP2, F₂-IsoP3, and F₂-IsoP4) were quantified using liquid chromatography/ tandem mass spectrometry and adjusted for urinary creatinine levels. Hypertension was assessed at baseline and follow-up visits and defined as systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg and/or currently taking antihypertensive medications.

Crude associations between study population characteristics and hypertensive status were analyzed with the chi-square and Wilcoxon-rank sum tests. Crude associations between study population characteristics and F₂-isoprostane levels were analyzed with Wilcoxon-rank sum, Kruskal-Wallis, and Spearman's rank correlation measures. Finally, the adjusted prospective associations between hypertensive status and F₂-isoprostane concentrations were modeled using logistic regression.

Results: Of the 844 participants who were included in the study, 258 (31%) were classified as hypertensive at baseline. Among the 586 participants who were normotensive at baseline, 123 (21%) developed hypertension over the five-year study period. Importantly, none of four F₂-isoprostane isomers predicted a significant increase in the odds of developing hypertension, as indicated by their odds ratio 95% confidence intervals; F₂-IsoP1: (0.85, 1.31), F₂-IsoP2: (0.62, 1.13), F₂-IsoP3: (0.80, 1.27), and F₂-IsoP4: (0.84, 1.29).

Conclusion: Previous studies have investigated the association between oxidative status and hypertension prevalence, however the cross sectional nature of the study designs have made it difficult to establish temporality between exposure and outcome. To our knowledge, this is the first study to model the odds of developing hypertension as a function of F₂-isoprostane levels. The results of this study suggest that oxidative status is not involved in the development of hypertension.

Table of Contents

Chapter 1:	
Introduction	1
1.1 Background.....	1
1.2 Purpose of Study	1
Chapter 2:	
Literature Review	2
2.1 Hypertension	2
2.2 Epidemiology of Hypertension	2
2.3 Risk Factors for Hypertension	3
2.4 Reactive Oxygen Species and Oxidative Stress.....	4
2.5 Measuring Oxidative Stress	6
2.6 F ₂ -Isoprostanes.....	7
2.7 Oxidative Status and Hypertension	8
Chapter 3:	
Methods.....	9
3.1 Data Source	9
3.2 Case Ascertainment.....	9
3.3 Covariates.....	10
3.4 Assessment of Main Exposure: Urinary F ₂ -Isoprostanes.....	10
Chapter 4:	
Statistical Analysis	11
4.1 Main Exposure.....	11
4.2 Crude Associations: Hypertension Status and Covariates	11
4.3 Crude Associations: F ₂ -isoprostanes and Covariates	12
4.4 Adjusted Associations: Hypertension Status and F ₂ -isoprostanes	12
Chapter 5:	
Results.....	13
5.1 Crude Associations: Hypertension Status and Covariates	13
5.2 Crude Associations: F ₂ -isoprostanes and Covariates.....	15
5.3 Adjusted Associations Hypertension Status and F ₂ -Isoprostanes	17
Chapter 6:	
Discussion.....	19
6.1 Primary Findings	19
6.2 Secondary Findings.....	20
6.3 Conclusion	21
Bibliography	23

Chapter I

Introduction

1.1 Background

Hypertension, a pathological condition of the cardiovascular system, affects one out of every three adults in the United States and its prevalence is expected to increase 7.2% by year 2030 (1). Hypertension is also a primary antecedent of cardiovascular disease, which is ranked first in the cause of death worldwide (2). When an elevated blood pressure persists and cannot be attributed to a specific cause, such as renal disease, it is diagnosed as *essential* or *primary* hypertension (3). Essential hypertension affects a majority of hypertensive cases and a complete understanding of its etiology remains unknown (4). There is increasing evidence that suggests oxidative stress plays a causal role in the pathogenesis of many chronic diseases, including hypertension (5). Importantly, hypertension is a modifiable risk factor. Thus, if a relationship between oxidative stress and hypertension were established, this would provide a foundation for prevention.

1.2 Purpose of Study

The primary objective of this study is to assess the adjusted prospective association between F₂-isoprostanes, a biomarker of oxidative status, and development of hypertension in a large cohort. Crude associations between F₂-isoprostanes, a set of demographic and anthropometric variables, and hypertensive status will also be assessed in order to identify potential confounding relationships.

Chapter 2

Literature Review

2.1 Hypertension

Hypertension is a chronic disease of the cardiovascular system characterized by a pathologic elevation of arterial blood pressure (6). Left uncontrolled, hypertension can promote damage to various organs and increase the risk of cardiovascular events including stroke, aneurysm, and ischemia (3). Blood pressure is a function of cardiac output and peripheral resistance, and it is believed that alterations in peripheral resistance contribute substantially to a hypertensive state (4). Hypertension is commonly diagnosed by averaging respectively several systolic (SBP) and diastolic blood pressure (DBP) measurements taken on different occasions (3). The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure defines hypertension as a DBP ≥ 90 mm Hg or SBP ≥ 140 mm Hg (7). The majority of hypertensive cases are classified as *idiopathic* or *essential* hypertension, in which there is no clinically identifiable cause (6). In addition, hypertension does not display any outward signs, thus it is commonly referred to as “the silent killer”(8).

2.2 Epidemiology of Hypertension

Hypertension is one of the most commonly diagnosed conditions in the world (9). In the U.S. alone, it affects approximately 1/3 of the population (10). In the past decade, the U.S. has experienced a 41.5% increase in the number of deaths due to

hypertension. Many treatments have been designed to regulate blood pressure; however, only 46% of hypertensive individuals have this condition under control (1). It is estimated that 83% of individuals with untreated hypertensive will die of ischemic heart disease or stroke (11). Hypertension also consumes valuable resources. In 2010, essential hypertension was involved in over 43 million medical care visits. In the same year, the health care cost of hypertension was an estimated \$46 billion, and projections suggest an increase to \$274 billion by 2030 (1). Despite the efforts of several national initiatives to improve hypertension prevention, detection, and treatment, there is an urgent need for continued research in the pathogenesis of hypertension and its deadly sequelae.

2.3 Risk Factors for Hypertension

Hypertension is believed to be a multifactorial condition influenced by genetics, environment, and behaviors (12, 13). In fact, researchers estimate that 30%-60% of blood pressure variability can be explained by genetics (14). The distribution of hypertension in the population is influenced by many characteristics including age, race, geographic location, gender, and socio-economic status (3). Lifestyle choices such as alcohol intake (15) and poor diet (16) have also been linked to hypertension. Hypertension prevalence is disproportionately high in the southeastern United States (17). Some reasons for this include a greater proportion of African Americans, decreased physical activity, and increased sodium intake.

Hypertension is often associated with a cluster of metabolically related conditions, namely dyslipidemia, glucose intolerance, and abdominal obesity (18, 19).

Data from the Framingham Heart Study suggests that with respect to each of these conditions, hypertension occurs independently of the other three about 20% of the time (20). It is believed that elevated levels of reactive oxygen species (ROS) mediate the relationship between obesity, insulin resistance, and hypertension (21). Experimental evidence suggests that a pathologic change in the renin angiotensin system, which is responsible for blood pressure regulation, is closely linked to obesity (22, 23). It has also been hypothesized that decreased physical activity and over-nutrition is responsible for free fatty acid and glucose overload in cells, which results in pathologic imbalances in ROS levels (24).

2.4 Reactive Oxygen Species and Oxidative Stress

ROS are a group of highly reactive molecules containing oxygen and found in all aerobic organisms (25). ROS are constantly produced by normal cellular processes and are involved with many functions including pathogen defense, signaling pathways, and elicitation of mitogenic responses (26).

Within the cardiovascular system, ROS such as superoxide and hydrogen peroxide play an important role in endothelial function, vascular tone, and cardiac function (27). Redox signaling pathways, which rely on temporary imbalances between pro-oxidant and antioxidant molecules, modulate production of nitric oxide (NO), which in turn controls vascular tone (28, 29). Within the vasculature, cytokines and hormones such as angiotensin-II, endothelin-I, and urotensin-II stimulate the production of superoxide and other ROS via NADPH oxidase activation (30). Because ROS play an integral role in cellular functions involved with blood pressure regulation, researchers

have hypothesized that significant and sustained disturbances in the reduction-oxygen (redox) balance drive the pathology of hypertension and other cardiovascular pathologies (31).

Redox signaling relies on a temporary imbalance between pro-oxidant and antioxidant molecules (25). In fact, evolution has equipped all aerobic organisms with an elaborate antioxidant defense system to maintain a steady state redox balance. By scavenging free radicals, enzymatic antioxidants such as superoxide dismutase and glutathione peroxidase protect cellular components against oxidative damage (27). However, prolonged increases in ROS and/or decreases in antioxidant capacity may cause cellular damage or interrupt normal cellular functioning.

Oxidative stress occurs when there is a significant imbalance between ROS production and antioxidant defense within the cells and tissues (32). In contrast to the temporary fluctuations in redox balance that drives cellular signaling, a systemic and chronic overabundance of ROS can react with essential biological molecules, changing their structure and function. Cellular components such as proteins, DNA, and lipids are frequent targets of ROS attack (31).

Several lines of evidence connect the over-stimulation of ROS generating enzymes and subsequent increases in ROS levels to hypertensive alterations in the cardiovascular system. Certain genetic, hormonal, and hemodynamic factors are believed to be responsible for the over-activation of NADPH oxidase, resulting in excessive production of ROS including superoxide, hydrogen peroxide, and peroxynitrite (32). An overabundance of these reactive species is believed to decrease NO

bioavailability within vascular tissue, leading to endothelial dysfunction, reduced vasodilation, and increased vasoconstriction (28, 33). Specifically, superoxide production may inhibit prostacyclin formation and accelerate breakdown of nitric oxide, an inhibitor of platelet aggregation (34). Angiotensin-II, a potent vasoconstrictor, directly influences blood pressure and is known to stimulate superoxide production (35, 36). Oxidative stress has also been shown to indirectly promote platelet activation, cell adhesion, and inflammatory responses within blood vessels by disrupting the thromboxane receptor interaction with its ligand TxA₂ (37). Importantly, endothelial dysfunction and inflammation may perpetuate additional ROS production, thus establishing a feedback cycle between the initial factors and the hypertensive state (29).

Pathogenic increases in oxidative molecules may also occur in other tissues responsible for blood pressure regulation. An over-activation of ROS-generating enzymes have been implicated in the alteration of redox signaling in kidney cells, which may promote glomerular damage and increased sodium and H₂O retention (13, 14). It has also been hypothesized that elevated levels of ROS within the hypothalamus may alter sympathetic outflow and baroreceptor reflex, leading to increase in blood pressure (32).

2.5 Measuring Oxidative Stress

ROS are reactive and unstable byproducts of oxygen metabolism (25). As such, they are difficult to quantify. Accordingly, researchers have focused their attention on *oxidative status*, which is used to characterize the relative state of oxidative load by measuring oxidative damage to biological molecules (38). The accepted method of

measuring oxidative status is via non-enzymatically formed biomarkers of oxidative damage (39). It is important to note that biomarkers of oxidative status capture the extent of damage caused by ROS, not ROS itself. However, the amount of oxidative damage is assumed to be proportional to the systemic levels of ROS that are not captured by the antioxidant defense, thus presenting a balance between generation and elimination of ROS at a systemic level (38). However, because ROS are ubiquitous and play many functional roles in aerobic organisms, it remains unknown which levels of oxidative status are considered to signify harmful oxidative stress as opposed to physiologically normal levels.

2.6 F₂-Isoprostanes

F₂-isoprostanes are a group of bioactive compounds formed by the free radical-mediated peroxidation of arachidonic acid, a lipid found within cellular membranes (40). Extensive research has shown that F₂-isoprostanes are valid and reliable markers of oxidative status in animals and humans (38). F₂-isoprostanes present biomarkers suitable for epidemiological research because these molecules are chemically stable and display high inter-individual and low intra-individual variation (41). Importantly, their generation is not influenced by diet (42, 43). F₂-isoprostanes can be quantified in bodily fluids using non-invasive methods and they have been used in both clinical and epidemiological studies. Because F₂-isoprostanes present an indices of the overall oxidative status, they may be a valuable tool in predicting pathological cardiovascular states and elucidating physiological processes involved with the development of adverse cardiovascular functioning, such as hypertension (44, 45).

2.7 Oxidative Status and Hypertension

There is a growing body of epidemiological inquiry applying F₂-isoprostane quantification to hypertension research. Some of these studies have reported significant increases in F₂-isoprostanes and other lipid peroxidation by-products among hypertensive cases compared to normotensive controls (46-49). Researchers have also observed elevated levels of hydrogen peroxide and superoxide radicals and decreased concentrations of antioxidants including superoxide dismutase and alpha-tocopherol among hypertensive cases (50, 51). In contrast, other studies have yielded no significant associations between F₂-isoprostanes and hypertension status (52, 53). Importantly, all of the previously published studies are cross sectional, thus temporal relationships between the exposure and outcome cannot be delineated.

Taking into account strong biological plausibility of ROS involvement with dysfunction in various tissues that regulate blood pressure, and lack of information about prospective associations between F₂-isoprostanes and incident hypertension, this study examined whether a prospective relationship exists between F₂-isoprostanes and hypertension incidence.

Chapter 3

Methods

3.1 Data Source

This analysis utilized existing data from the Insulin Resistance Atherosclerosis Study (IRAS). IRAS is a prospective epidemiological study designed to assess the relationships between insulin resistance, type-two diabetes, cardiovascular disease, and other risk factors among a multi-centered sample of non-Hispanic white, Hispanic, and African American individuals (54). 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 racial/ethnic and geographic diversity, the IRAS study aimed to recruit participants who were metabolically diverse. The sampling methodology ensured adequate representation of groups with normal and impaired glucose tolerance in addition to type-two diabetes.

3.2 Case Ascertainment

Each participant's hypertensive status was evaluated at baseline and 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 2nd and 3rd measurements were used in determining hypertensive status. 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.

3.3 Covariates

The participants were followed for approximately five years. Baseline and follow-up examinations were each conducted during two visits, separated by one week. Before each visit, participants were requested to fast for 12 hours and abstain from alcohol, smoking, and heavy exercise. All participants completed an extensive examination that assessed many demographic, lifestyle, and anthropometric variables. Age, gender, race/ethnicity, and smoking status were self-reported and captured using validated questionnaires. The analytical cohort included non-diabetic participants at baseline as determined by the oral glucose tolerance test. Impaired glucose tolerance was assessed at baseline and follow-up visits using a 75g oral glucose tolerance test. Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters for each participant and represented overall adiposity.

3.4 Assessment of Main Exposure: Urinary F₂-Isoprostanes

During the baseline examination, morning spot urine samples were taken from all participants and stored at -70°C. F₂-isoprostanes were quantified using liquid chromatography/tandem mass spectrometry and adjusted for urinary creatinine concentration. A total of four isomers [(iPF(2α)-III), (2,3-dinor-iPF(2α)-III), (iPF(2α)-IV), (8,12-iso-iPF(2α)-IV)] were evaluated. After excluding individuals with baseline diabetes and those with missing values on any variable from either baseline or follow-up examinations due to loss of follow-up or technical complications, we included 844 non-diabetic participants in the current analysis.

Chapter 4

Statistical Analysis

4.1 Main Exposure

In the presented analysis, the four F₂-isoprostane variants [(iPF(2 α)-III), (2,3-dinor-iPF(2 α)-III), (iPF(2 α)-IV), (8,12-iso-iPF(2 α)-IV)] will be referred to as F₂-isoP1, F₂-isoP2, F₂-isoP3, F₂-isoP4, respectively. Additionally, an F₂-isoprostane index (F₂-isoP index) variable was created by calculating the standardized mean of the four isomers. Lastly, principle components analysis, which is a variable reduction technique, was conducted on the original F₂-isoprostane isomers in order to identify a smaller set of factors that would explain a majority of the shared variance between all four isomers, yet exhibit no correlation/co-linearity when included together in a linear model of hypertension. Principle component analysis resulted in identification of two unique factors that collectively explained approximately 80 % of the shared variance between F₂-isoP1, F₂-isoP2, F₂-isoP3, and F₂-isoP4. These factors were included together in the logistic regression model of hypertension discussed later in the study.

4.2 Crude Associations: Hypertension Status and Covariates

The examination of unadjusted associations between prevalent hypertension and categorical variables was carried out using X^2 tests. Similarly, the crude associations between categorical study characteristics and incident hypertensive cases were assessed using X^2 tests. In addition, the Wilcoxon-rank sum test was used to

assess whether BMI and F₂-isoP levels differed between those with and without prevalent and incident hypertension.

4.3 Crude Associations: F₂-isoprostanes and Covariates

In order to compare F₂-isoP levels among the strata of a categorical variable, this study utilized the Wilcoxon-rank sum and Kruskal-Wallis test. The Spearman correlation coefficient was used to examine the associations between F₂-isoPs and continuous variables.

4.4 Adjusted Associations: Hypertension Status and F₂-isoprostanes

This study included a cross-sectional and a prospective analysis to assess the relationship between hypertension status and the main exposure, urinary F₂-isoP. Logistic regression modeling was performed to assess adjusted cross-sectional and prospective associations between hypertension status and F₂-isoP. Fully adjusted and minimally adjusted models were included in both analyses. The minimally adjusted model included age (years), sex, ethnicity (African-American/non-Hispanic white/Hispanic), clinic location (four strata), and BMI (kg/m²). The fully adjusted model included two additional variables: smoking status (never/former/current) and IGT status (normal/ impaired glucose tolerance). The adjusted odds ratios for continuous covariates and F₂-isoP predictors were scaled by their respective standard deviations. With the exception of the two F₂-isoP factors created by principle component analysis, all of the original F₂-isoP variables and the standardized index were included separately in each logistic regression model. This was done to prevent statistical issues that can

arise from co-linearity between predictors. The statistical analysis was performed using the SAS software package (version 9.3; SAS Institute, Cary N.C.). All statistical results were assessed at the p -value <0.05 .

Chapter 5

Results

5.1 Crude Associations: Hypertension Status and Covariates

At baseline (Table 1), 30.6% of the study population was classified as hypertensive. The normotensive group was approximately five years younger than their hypertensive counterpart ($p<0.001$). There also existed a significant difference in the proportion of hypertensive cases with respect to race/ethnicity ($p<0.001$). Among African-Americans, 40.6% were classified as hypertensive baseline. In contrast, only 26% of whites and 28% of non-white Hispanics were hypertensive at baseline. Smoking was not associated with prevalent hypertension in this study population. There was a significant difference in the proportion of hypertensive cases with respect to IGT-status ($p<0.0001$). Among the participants with normal glucose tolerance, only 26% were hypertensive, while 41% of individuals with impaired glucose tolerance were hypertensive. Hypertensive individuals on average had greater BMI as compared to normotensive ($p=0.0001$). The normotensive and hypertensive groups differed by varying degrees of significance with respect to the four F_2 -isoP concentrations. Interestingly, F_2 -isoP1 ($p=0.04$) and F_2 -isoP3 ($p=0.001$) were lower among individuals with a normotensive status at baseline.

Table 1. Study characteristics and hypertension status

Characteristics	Cross Sectional		<i>p</i> *	Prospective		<i>p</i> *
	Normotensive At Baseline n= 586	Hypertensive At Baseline n= 258		Normotensive At Follow-up n= 463	Hypertensive At Follow-up n= 123	
Age	53 ± 8.2	58 ± 7.7	<0.001	53 ± 8.1	54 ± 8.6	0.1
Gender (%)			0.9			0.03
Male	250 (42.7)	110 (42.6)		187 (40.4)	63 (51.2)	
Female	336 (57.3)	148 (57.4)		276 (59.6)	60 (48.8)	
Ethnicity (%)			<0.001			0.05
White	250 (42.7)	88 (34.1)		200 (43.2)	50 (40.7)	
African American	139 (23.7)	95 (36.8)		100 (21.6)	39 (31.7)	
Non-white Hispanic	197 (33.6)	75 (29.1)		163 (35.2)	34 (27.6)	
IGT Status (%)			< 0.0001			0.1
Normal	424 (72.4)	146 (56.6)		342 (73.9)	82 (66.7)	
IGT	162 (27.7)	112 (43.4)		121 (26.1)	41 (33.3)	
Smoking Status (%)			0.1			0.08
Never	273 (46.6)	121 (46.9)		223 (48.2)	50 (40.7)	
Past	221 (37.7)	110 (42.6)		164 (35.4)	57 (46.3)	
Current	92 (15.7)	27 (10.5)		76 (16.4)	16 (13)	
BMI	27.6 ± 5.2	30.2 ± 6.1	0.0001	27.37 ± 5.07	28.58 ± 5.73	0.03
F ₂ -isoP1	0.25 ± 0.18	0.24 ± 0.2	0.04	0.25 ± 0.18	0.24 ± 0.23	0.1
F ₂ -isoP2	4.29 ± 3.07	4.47 ± 2.84	0.4	4.39 ± 3.21	3.86 ± 2.46	0.02
F ₂ -isoP3	6.7 ± 4.14	6.01 ± 4.2	0.001	6.76 ± 4.06	6.48 ± 4.44	0.1
F ₂ -isoP4	4.17 ± 2.76	4.08 ± 3.11	0.2	4.19 ± 2.78	4.07 ± 2.66	0.6
F ₂ -isoP Index	0.01 ± 0.8	-0.04 ± 0.86	0.07	0.03 ± 0.79	-0.06 ± 0.84	0.09

*Categorical variables were reported as *n*, (%) and assessed using Chi Square test

Continuous variables were reported as mean ± SD but assessed using Wilcoxon Rank Sum/ Kruskal Wallis test

Among those who were classified as normotensive at baseline (*n* = 586), 21% developed hypertension during the five-year study period (Table 1). Unlike the cross-sectional analysis, there was no significant difference between incidence hypertension cases and non-cases with respect to age. However, the proportion of males who developed hypertension (25.2%) was significantly greater (*p*=0.03) as compared to the proportion of females developing hypertension (17.9%). With respect to race/ethnicity, there was a marginally significant difference between the proportions of those who developed hypertension (*p*=0.05). Similar to the cross sectional analysis, the African

American stratum exhibited a greater proportion of individuals who developed hypertension (28%) compared to white (20%) and non-white Hispanic (17%) strata. However, there was no significant difference between hypertension incidence with respect to IGT-status or smoking. Median baseline BMI among the cases was lower ($p = 0.03$) compared to their normotensive counterparts. Among the five F₂-isoP measurements, only F₂-isoP2 ($p=0.02$) differed significantly between the normotensive (4.39 ± 3.21 ng/mg creatinine) and hypertensive group (3.86 ± 2.46 ng/mg creatinine).

5.2 Crude Associations: F₂-isoprostanes and Covariates

To assess the association between characteristics of the study population and oxidative damage, each stratum of a given categorical variable were compared with respect to median F₂-isoP (Table 2). Females consistently displayed greater levels of all five F₂-isoP ($p \leq 0.002$). Among the race/ethnicity strata, Hispanics showed greater levels of all five F₂-isoP measurements, while African-Americans displayed the lowest concentrations. Impaired glucose tolerance was not consistently associated with F₂-isoP levels and showed a significant increase ($p=0.03$) in only F₂-isoP2. Additionally, there were significant differences in F₂-isoP1 ($p<0.0001$), F₂-isoP3 ($p=0.02$), and the F₂-isoP index ($p<0.001$) with respect to smoking status. While smokers displayed greater F₂-isoP, past smokers typically displayed the lowest levels of F₂-isoP.

Significant inverse associations were observed between age and two F₂-isoP measurements: F₂-isoP4 ($p<0.001$) and the F₂-isoP index ($p=0.04$). Finally, there was a significant positive association between BMI and F₂-isoP2 ($p<0.001$), F₂-isoP4 ($p< 0.004$), and the F₂-isoP index ($p=0.007$).

Table 2. Associations between F₂-IsoPs (ng/mg creatinine) and study characteristics

		F ₂ -IsoP1 [iPF(2 α)-III]	F ₂ -IsoP2 [2,3-dinor- iPF(2 α)-III]	F ₂ -IsoP3 [iPF(2 α)-IV]	F ₂ -IsoP4 [8,12-iso- iPF(2 α)-IV]	F ₂ -IsoP Index
Categorical demographic and baseline characteristics; mean (s.d.)						
Gender	Male	0.19 (0.16)	3.19 (1.85)	5.01 (3.12)	3.77 (2.44)	-0.29 (0.62)
	Female	0.29 (0.2)	5.19 (3.39)	7.59 (4.49)	4.42 (3.12)	0.21 (0.88)
	<i>p</i> -value ^a	P<0.0001	P<0.001	P<0.001	P=0.002	P<0.001
Ethnicity	Non-Hispanic White	0.24 (0.17)	4.08 (2.29)	6.24 (3.74)	4.13 (2.65)	-0.05 (0.69)
	African American	0.19 (0.15)	3.59 (2.06)	5.08 (2.98)	3.26 (2.03)	-0.31 (0.56)
	Hispanic	0.31 (0.24)	5.31 (4.04)	8.02 (4.99)	4.93 (3.47)	0.32 (1.02)
	<i>p</i> -value ^a	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001
IGT Status	Normal	0.25 (0.21)	4.19 (3.01)	6.42 (3.99)	4.09 (2.91)	-0.02 (0.83)
	IGT	0.24 (0.16)	4.64 (2.96)	6.65 (4.52)	4.24 (2.78)	0.03 (0.79)
	<i>p</i> -value ^a	P=0.55	P=0.03	P=0.94	P=0.28	P=0.34
Smoking Status	Never	0.24 (0.18)	4.34 (2.69)	6.75 (4.36)	4.2 (2.83)	0.01 (0.79)
	Past	0.23 (0.16)	4.17 (3.27)	6.08 (4.05)	3.94 (2.64)	-0.09 (0.79)
	Current	0.34 (0.27)	4.83 (3.18)	6.76 (3.76)	4.53 (3.51)	0.19 (0.96)
	<i>p</i> -value ^a	P<0.0001	P=0.05	p=0.02	P=0.15	P<0.001
Continuous anthropometric baseline characteristics. Spearman correlation coefficients (<i>p</i> -value)						
Age		-0.01 (0.7)	-0.03 (0.3)	-0.03 (0.4)	-0.19 (<0.001)	-0.07 (0.04)
BMI		0.0 (0.9)	0.18 (<0.001)	0.04 (0.3)	0.1 (0.004)	0.09(0.007)

^a Wilcoxon rank sum/ Kruskal- Wallis test

5.3 Adjusted Associations Hypertension Status and F₂-Isoprostanes

The minimally and fully adjusted odds of prevalent hypertension (Table 3) were calculated using two sets of covariates as defined in the Methods section. Overall, there was no consistent association between F₂-isoP and hypertension prevalence, as the OR point estimates ranged from 0.81 to 1.05 in the reduced model and 0.82 to 1.05 in the full model. However, a marginal inverse association was found between F₂-IsoP3 and hypertension prevalence in the reduced (C.I. 95%=0.67,0.99) and the full model (C.I. 95%= 0.68,0.99).

Table 3. Multivariable logistic regression of baseline hypertension on F₂-isoprostanes

F ₂ -isoprostanes (ng/mg creatinine)	Baseline Hypertension (n=258)	
	OR ^a (95%CI)	OR ^a (95% CI)
	Model 1 ^b	Model 2 ^c
F ₂ -IsoP1 [iPF(2 α)-III]	0.99 (0.83,1.19)	1.02 (0.85,1.23)
F ₂ -IsoP2 [2,3-dinor-iPF(2 α)-III]	0.98 (0.83,1.16)	0.99 (0.83,1.17)
F ₂ -IsoP3 [iPF(2 α)-IV]	0.81 (0.67,0.99)	0.82 (0.68,0.99)
F ₂ -IsoP4 [8,12-iso-iPF(2 α)-IV]	1.05 (0.88,1.24)	1.05 (0.88,1.25)
F ₂ -isoP Index	0.94 (0.78,1.14)	0.96 (0.79,1.15)
Factor 1	0.95 (0.79, 1.16)	0.92 (0.77, 1.10)
Factor 2	1.01 (0.86, 1.18)	0.99 (0.84, 1.16)

CI, confidence interval; OR, odds ratio

^a Odds ratios scaled by respective standard deviation

^b Reduced model adjusted for the following variables: age, gender, race/ethnicity, clinic, BMI

^c Full model adjusted for the following variables: age, gender, race/ethnicity, clinic, smoking status, IGT status, BMI

In a similar manner, the associations between F₂-isoP and incident hypertension were examined (Table 4). In both the fully and minimally adjusted models, none of F₂-isoPs predicted a significant change in the odds of incident hypertension. These results do not support the theory that increased oxidative status is causally related to hypertension.

Table 4. Multivariable logistic regression of incident hypertension on F₂-isoprostanes

F ₂ -isoprostanes (ng/mg creatinine)	Hypertension at follow-up (n=463)	
	OR ^a (95%CI)	OR ^a (95% CI)
	Model 1 ^b	Model 2 ^c
F ₂ -IsoP1 [iPF(2 α)-III]	1.06 (0.85, 1.31)	1.08 (0.86,1.36)
F ₂ -IsoP2 [2,3-dinor-iPF(2 α)-III]	0.84 (0.62, 1.13)	0.84 (0.62, 1.13)
F ₂ -IsoP3 [iPF(2 α)-IV]	1.01 (0.80, 1.27)	1.0 (0.79,1.27)
F ₂ -IsoP4 [8,12-iso-iPF(2 α)-IV]	1.04 (0.84, 1.29)	1.05 (0.85,1.31)
F ₂ -isoP Index	0.99 (0.78, 1.26)	1.0 (0.8, 1.3)
Factor 1	0.96 (0.74, 1.25)	0.88 (0.69, 1.12)
Factor 2	0.81 (0.63, 1.06)	0.77 (0.60, 1.01)

CI, confidence interval; OR, odds ratio

^a Odds ratios scaled by respective standard deviation

^b Reduced model adjusted for the following variables: age, gender, race/ethnicity, clinic, BMI

^c Full model adjusted for the following variables: age, gender, race/ethnicity, clinic, smoking status, IGT status, BMI

Chapter 6

Discussion

6.1 Primary Findings

Using the cross-sectional and prospective designs, this analysis investigated the association between urinary F₂-isoP and hypertension status in a large cohort with demographic, anthropometric, and metabolic diversity. This study produced two main findings. First, the analysis could not establish a significant relationship between increased oxidative status and hypertension prevalence. After controlling for a minimal set of potential confounders, the ORs associated with seven F₂-isoP measurements ranged from 0.81 to 1.05. Similarly, the range of the fully adjusted ORs ranged from 0.82 to 1.05. These findings are consistent and in agreement with previously published studies that found no evidence of increased F₂-isoP levels among the prevalent hypertensive cases (52, 53).

With respect to incident hypertension, to the best of our knowledge, this is the first study to conduct a prospective study between F₂-isoP levels and hypertension. The minimally and fully adjusted odds ratios ranged from 0.81 to 1.06 and from 0.77 to 1.08, respectively, with none of the associations reaching statistical significance. These results suggest that increased oxidative status, as measured by F₂-isoP, is not causally related to hypertension pathogenesis. Other lines of evidence support this reasoning. First, it has been shown that ROS generation occurs because of endothelial dysfunction (55). One study also showed that men treated with anti-hypertensive medication exhibited lower concentrations of F₂-isoP compared to untreated controls (53).

Additionally, large clinical trials of antioxidants have shown inconsistent results in reducing blood pressure (56).

Interestingly, two sets of crude associations also contradict the original hypothesis that increased oxidative status is casually associated with hypertension. African-Americans exhibited the lowest baseline concentrations of F₂-isoP, yet also exhibited the greatest proportion of hypertensive cases at baseline and follow-up. If the original hypothesis were true, we would expect to see a direct association between the primary exposure and outcome.

6.2 Secondary Findings

Importantly, several expected associations between known risk factors and hypertension have been found. For example, this study found that age differed significantly between baseline normotensives and their hypertensive counterparts. Accordingly, age is a known risk factor for hypertension and cardiovascular disease (12). Interestingly, this association was not present in the crude prospective association. One reason for this may be a lack of age variability in the sub-group who were normotensive at baseline. While previous studies found a positive association between F₂-isoP and age (5), this analysis found significant inverse associations between age and two of the four F₂-isoP measurements. One possible explanation for this association is that oxidative metabolism capacity declines with age. Additionally, this study found expected crude associations between hypertension status and race/ethnicity (57), IGT status (18), and BMI (58). Although positive associations between hypertension and F₂-isoP concentrations have been found in previous cross-sectional studies (46, 47, 49, 59),

the cross-sectional associations were marginally significant in this study, suggesting a potential protective trait of increased oxidative status. Indeed, some researchers theorize that an increased concentration of F₂-isoPs reflect a favorable trait and infers a reduced risk of weight gain and development of diabetes (60).

The unadjusted associations between baseline study characteristics and F₂-isoP species produced several expected findings. F₂-isoP levels differed significantly between males and females. However, this may be explained by the adjustments made for urinary diluteness during quantification of urinary biomarkers. Specifically, corrections for creatinine levels were made to all F₂-isoP concentrations. Since creatinine levels are influenced by the lean muscle mass, these adjustments can increase observed concentrations among women, because women on average have lower muscle as compared to men (60). This study also reproduced associations between F₂-isoP and smoking status (41) and BMI (58).

6.3 Conclusion

Despite advances in prevention and treatment, hypertension is still one of the most common conditions in the world, and for a majority of cases the causal mechanisms remain to be fully understood. Many experimental studies have implicated oxidative stress in the pathogenesis of hypertension. However, epidemiological studies have not provided consistent results. Additionally, most of the epidemiological studies are cross sectional and thus, cannot establish temporal relationships between the exposure and outcome. The main objective of this study was to investigate the prospective relationship between oxidative status, as measured by F₂-isoP, and

hypertension incidence. The results of this study do not support the hypothesis that elevated oxidative status can inform hypertension risk. While this study implemented the prospective design to establish a temporal relationship between oxidative stress status and hypertension there were several inherent limitations. These include dichotomization of hypertension and a lack of repeated blood pressure and F₂-isoP observations during the five-year study period. Additionally, oxidative status must be assessed indirectly through oxidative damage. Since there are multiple biological pathways involved with hypertension, additional measures of oxidative damage may provide a more comprehensive assessment of oxidative stress status.

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