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Does Oxidative Status Predict Progress in Subclinical Atherosclerosis: An Analysis of the Insulin Resistance Atherosclerosis Study

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Does Oxidative Status Predict Progress in Subclinical Atherosclerosis: An Analysis of the
Insulin Resistance Atherosclerosis Study

by

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BSc., University of Pittsburgh

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of Georgia State University in Partial Fulfillment
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APPROVAL PAGE

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Insulin Resistance Atherosclerosis Study

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Abstract

Purpose

There has been a strong biological plausibility for a causal role of reactive oxygen species in cardiovascular disease pathology. However, randomized clinical trials of antioxidants showed no association between antioxidant intake and reduced risk of CVD mortality nor morbidity. To date, there has been no direct epidemiological evidence linking oxidative status to subclinical atherosclerosis progression. We examined prospective relationships between oxidative status (urinary F2-Isoprostanes) and subclinical atherosclerosis (common/internal carotid intima-media thickness) in the Insulin Resistance Atherosclerosis Study cohort (N=857) to determine if elevated systemic levels of F2-isoprostanes are directly correlated with progression in subclinical atherosclerosis.

Methods

This study utilized data from the Insulin Resistance Atherosclerosis Study (IRAS), a prospective cohort study that was designed to examine the relationships between insulinemia, glycemia, insulin resistance, and cardiovascular risk in a racially and metabolically diverse population including African Americans, non-Hispanic whites, and Hispanics between 40-69 years of age at baseline²⁷. Between October 1992 and April 1994, 1625 men and women were recruited to participate in the study and followed up after 5 years²⁷. Four urinary F2-isoprostane isomers were quantified at baseline using liquid chromatography/tandem mass spectrometry and were summarized as a composite F2-isoprostane index, which was used as a marker for oxidative status. Linear and logistic regression were used to assess the relationship between F2-isoprostanes and the outcome of interest, subclinical atherosclerosis, measured by common carotid artery intima-media thickness (CCIMT) as well as internal carotid artery intima-media thickness (ICIMT). CCIMT and ICIMT were assessed at baseline and at the follow-up examinations. In logistic regression models, progression of CCIMT and ICIMT was defined as a >15% change during the follow-up examination and the main exposure was the baseline F2-isoprostane index. The covariates included age, sex, ethnicity and baseline BMI, smoking status, and systolic blood pressure (SBP). In linear regression models, natural log transformed CCIMT/ICIMT at follow-up were dependent variables and the baseline F2-isoprostane index was the main exposure with the baseline natural log transformed CCIMT/ICIMT and the same covariates included as predictors.

Results

After adjustment for age, sex, ethnicity, BMI status, smoking status and systolic blood pressure, logistic regression models showed that F2-isoprostanes had an inverse association with CCIMT progression (OR= 0.76, 95% CI [0.58, 1.01]) and no association with progression of ICIMT (OR = 0.94, 95% CI [0.76, 1.17]). Linear regression also showed a tendency of inverse relationships between F2-isoprostane index and change in CCIMT and ICIMT: beta-coefficients for F2-isoprostane index were -0.014, 95% CI (-0.029,0.0006)] and -0.0021, 95% CI (-0.030,0.026), in the models predicting change in CCIMT and ICIMT, respectively.

Conclusion

The result from this study suggest that elevated levels of F2-isoprostanes do not directly predict subclinical atherosclerosis progression. Future studies are needed to get a better understanding of the different forms of oxidative status markers and their influence on cardiovascular disease risk.

Author's Statement Page

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Shayla Williamson

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Introduction

Purpose of Study

Cardiovascular disease (CVD) is an umbrella term used to describe heart conditions that involve narrowed or blocked blood vessels mainly caused by atherosclerosis that can lead to adverse health outcomes such as heart attacks, strokes and angina¹. Cardiovascular disease is the leading cause of death in the United States accounting for 610,100, 1 out of 4, deaths every year². Understanding symptoms, indicators, and exposures increasing the risk of CVD is important for early detection and prevention. One way to detect early signs of CVD risk is to assess subclinical atherosclerosis. Subclinical atherosclerosis is an early indicator of atherosclerotic risk and timely recognition can slow or prevent progression to clinical manifestation of CVD³. Previously published studies suggested that oxidative stress promotes atherosclerosis and could be an indicator of increased risk for CVD⁴. Oxidative stress can be assessed using different biomarkers such as F2-isoprostanes, malondialdehyde, nitrotyrosine, S-glutathionylation, myeloperoxidase, oxidized LDL, and serum antioxidant capacity in relation to CVD. However, there is a gap in the literature on whether oxidative stress induces the progression of subclinical atherosclerosis. The aim for this study is to analyze the association between oxidative status assessed by urinary levels of F2-isoprostanes (oxidative status biomarker) and progression of subclinical atherosclerosis assessed by carotid intima-media thickness (biomarker for subclinical atherosclerosis). Our main research question is to determine whether elevated levels of urinary F2- isoprostanes predict increase in the carotid intima-media thickness. This study is a novel analysis with the goal to expand the current knowledge of the pathology of atherosclerosis with the intention of developing a better understanding of measurements for primary prevention of CVD.

Literature review

Atherosclerosis

Atherosclerosis, a specific type of arteriosclerosis, is a chronic-inflammatory disease that is defined by the narrowing of medium and large sized arteries due to the accumulation of fats and inflammatory cells²⁰. While the exact cause of atherosclerosis is not fully understood it is believed to be caused by various risk factors such as high blood pressure, high cholesterol, high triglycerides, smoking, inflammation, obesity, and diabetes⁷. According to Howard, et al. current smokers had a 50% increase in atherosclerosis progression rate compared to those that never smoked (43 μm vs 28.7 μm) while past smokers had a 25% increase in atherosclerosis progression compared to those that never smoked (35.8 μm vs 28.7 μm) assessed by common carotid intima-media thickness as a surrogate measure of atherosclerosis⁴⁶.

The factors mentioned previously can cause damage to the endothelium of the arteries and allow fat cells to enter into the artery wall⁷. On occasions, when the body tries to repair the injured site white blood cells, fats, and cholesterol clump together at the injury site and form plaque⁷. Eventually this plaque build-up can harden in the artery wall and start to restrict blood flow preventing oxygen and other important nutrients from reaching vital organs and tissues in the body, causing them to function poorly.

Atherosclerosis is a slow progressive disease that can start in childhood, but symptoms are usually not observed until middle or older age. Sometimes plaque build-up can break off from the artery wall and enter the blood stream. This can cause pain in the chest or in the legs when exercising oneself⁸. Other symptoms can include shortness of breath, fatigue, muscle weakness, and confusion however, not having symptoms is also common. A cross-sectional study with 262

participants with no signs or symptoms of atherosclerosis concluded that 52% had some sort of atherosclerosis, 85% of those 50 are older had atherosclerosis, and that it was present in 17% of children⁶¹. Due to the fact that there are sometimes no symptoms involved with atherosclerosis it is important to partake in prevention methods such as keeping track of blood pressure and cholesterol levels, eating healthy, exercising regularly, stop smoking, and decreasing alcohol intake to ensure decreased odds of getting atherosclerosis. Although there have been studies that showed decrease in plaque formation^{31,32,33}, Plaques usually do not go away after they are formed, so medications for high blood pressure and high cholesterol along with lifestyle changes are the best treatments to stop or slow the progression of plaque growth⁸. If plaque build-up is not properly managed atherosclerosis can lead to blood clots and cause cardiovascular events such as stroke, heart attacks, and angina. Due to this, it is important to detect sign of atherosclerosis as early as possible and one way to assess this is through subclinical atherosclerosis measurements.

Subclinical Atherosclerosis

Subclinical atherosclerosis is an early indicator of atherosclerotic risk before the manifestation of clinical symptoms and is believed to be an important tool for early detection of CVD risk⁶. Various techniques can be utilized to measure subclinical atherosclerosis. For example, arterial stiffness can be measured via pulse wave velocity, whereas other measures include ankle-brachial index (ABI), and carotid intima-media thickness (CIMT)⁶.

Arterial stiffness is the result of alterations of the arterial structure and function coupled with the deposition of collagen, calcium and loss of elastic matrix ⁶. Arterial stiffness has been

shown to play an important role in the progression of atherosclerosis and CVD. There is a variety of ways to measure arterial stiffness, but the most efficient marker was deemed to be pulse wave velocity which is the time required for the pressure wave to travel between two regions in the vasculature⁶.

Ankle-Brachial Index (ABI) is a ratio of the systolic blood pressure measured at the ankle to the systolic blood pressure in the upper arm. It is also a strong predictor for CVD events. ABI measurements less than 0.90 were shown to have a strong association with CVD events, HR, 1.20; 95% CI, 1.08 to 1.32⁶. Other studies have shown that having a low ABI compared to a normal ABI which is between 1.10-1.14 was an indication of a 2-3-fold increased risk of carotid atherosclerosis⁶.

Carotid artery intima-media thickness

(CIMT) is the measurement performed at the carotid arteries. Thickening of the arteries is due to the onset of atherosclerosis, the accumulation of fatty deposits and inflammatory cells in the walls of the arteries. These arteries carry oxygen rich blood from the heart to the head, face, and brain. CIMT can be measured either through the common carotid intima-media thickness (CCIMT) or the internal carotid intima-media thickness (ICIMT).

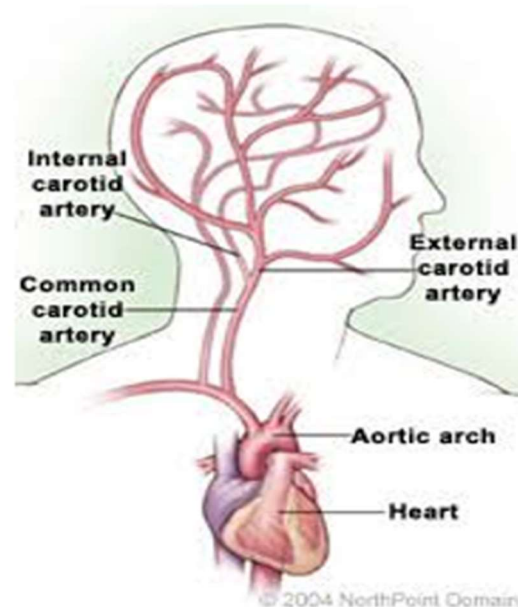


Figure 1. Branches of the Carotid Artery

The common carotid arteries are the ones that go from the heart to the neck on both sides and once they reach the neck, they branch off to form the internal carotid artery (inner side of the neck) and the external carotid artery (outer side of the neck). The external carotid artery supplies

blood to the face, scalp, and skull while the internal carotid artery supplies blood to the brain. The thickness of the two innermost layers of the carotid artery, called the tunica intima and tunica media, are used to detect atherosclerosis (Shown in Figure 2). Because the carotid arteries are large arteries close to the surface of the skin the two layers are able to be measured using non-invasive B-mode ultrasounds. Ultrasound measurements are usually taken at three different places, from the near and far walls of common carotid arteries, the carotid bifurcation, and the proximal internal carotid arterial segments⁴⁹. Once the ultrasound is complete the test will generate the measurements and a risk assessment⁴⁹.

The average CIMT is 0.40 to 0.50 mm for children and 0.70 mm or more for adults⁶. There have been some inconsistencies with regard to using CIMT as a predictor of CVD risk; however, the meta-analysis performed on previous studies showed that CIMT was associated with first myocardial infraction and

stroke along with other cardiovascular events⁶. However, to deal with these uncertainties it is hypothesized that CIMT combined with carotid plaque assessment is a better predictive model of

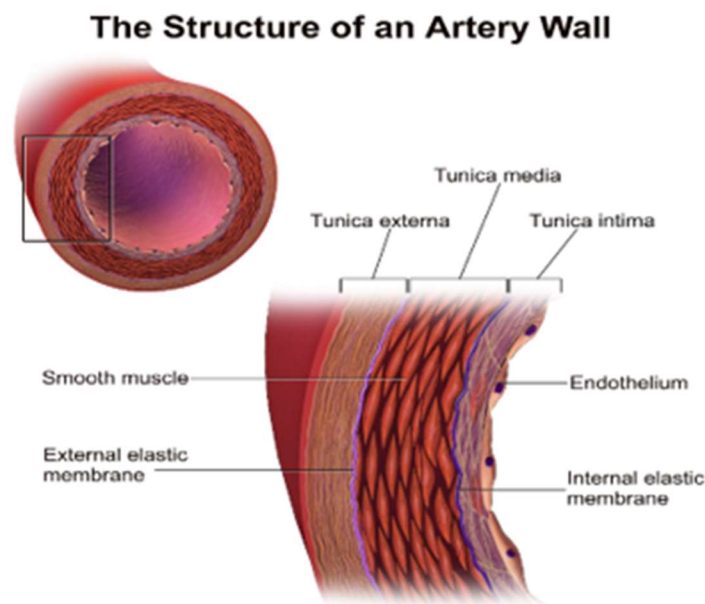


Figure 2. Structure of an artery wall

[Blausen.com staff \(2014\). "Medical gallery of Blausen Medical 2014". *Wiki.Journal of Medicine* 1 \(2\). DOI:10.15347/wjm/2014.010. ISSN 2002-4436.](#)

CVD events⁶. The most common location of atherosclerotic plaque buildup is in the inner wall of the carotid bifurcation area⁴⁹. Carotid plaques are detected through ultrasound and is defined as an area of the artery wall that is thicker than the surrounding CIMT area by a width of 0.5 mm or a CIMT greater than 1.5⁵⁰⁻⁵¹ as demonstrated in figure 3 below. One step that is believed to be important in the pathogenesis of atherosclerosis is the oxidation of low-density lipoproteins and understanding this mechanism may lead to discovering ways to prevent or slow atherosclerosis progression⁹.

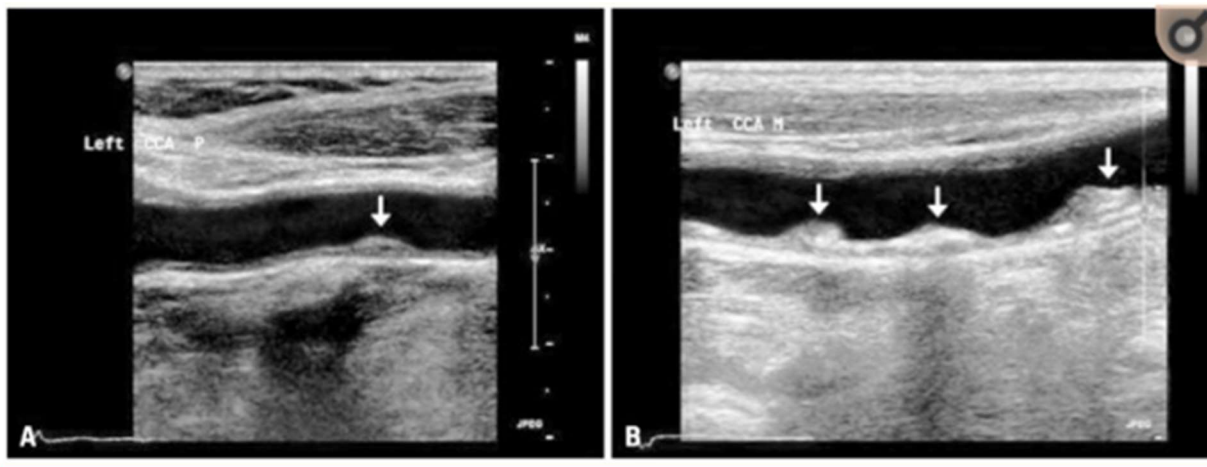


Figure 3. Demonstration of carotid artery plaques.

[Park T. H. \(2016\). Evaluation of Carotid Plaque Using Ultrasound Imaging. *Journal of cardiovascular ultrasound*, 24\(2\), 91–95. doi:10.4250/jcu.2016.24.2.91](#)

Lipid Peroxidation, F2-Isoprostanes, and Oxidative Status

Lipoprotein is a substance that is responsible for carrying cholesterol, triglycerides, and free fatty acids throughout the body¹⁰. There are four different classes of plasma lipoproteins substances, chylomicrons, very-low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoprotein (HDL)¹¹. HDL collects cholesterol from peripheral tissue, delivering cholesterol back to the liver for excretion¹². HDL is considered to be a beneficial

lipoprotein because it plays an important role in prevention of cardiovascular disease¹². Research has shown that high circulating HDL levels correlate with low rates of atherosclerosis¹². Gordon et al. showed that HDL is an independent cardiovascular risk factor and that the increase of HDL by only $10 \text{ mg}\cdot\text{L}^{-1}$ resulted in a risk reduction of 2–3%⁵². Di'Angelantonio et al. showed that those in the higher quantiles of HDL level had lower rates of coronary heart disease (CHD) per 1000 person years vs those in the lower quantiles for HDL levels (2.4 per 1000 p-yrs. vs 6.4 per 1000 p-yrs.) with a hazard ratio of 0.78 (95% CI, 0.74-0.82)⁵³. Additionally, Di'Angelantonio et al. examined LDL levels and saw that those with high circulating LDL levels had a higher rate of CHD per 1000 person years compared to those with low levels (6.7 per 1000 p-yrs. vs 2.3 per 1000 p-yrs.) with a hazard ratio of 1.50 (95% CI, 1.38-1.62)⁵³. These findings may be due to the antioxidant and anti-inflammatory properties seen in normal functioning HDL¹². Normal functioning HDL is known to prevent oxidation of LDL as well as prevent the inflammatory process seen after the oxidation¹².

Low-density lipoprotein, LDL, on the other hand is known to have a direct association with cardiovascular risk¹². LDL particles are formed through the hydrolysis of intermediate-density lipoprotein and contain a high level of cholesterol¹². LDL particles can be modified¹². One type of modification that is an important step in the pathogenesis of atherosclerosis, is oxidation of LDL, also called lipid peroxidation¹². The oxidation process can happen in many parts of the LDL however polyunsaturated fatty acids are the most susceptible constituents of LDLs due to the multiple double bonds within the structure. Lipid peroxidation of LDL is the result of a reaction between LDL and molecules called reactive oxygen species (ROS). ROS are a type of free radical mainly created by the mitochondria with the most common form being superoxide⁵³. The enzymes used in complex I, complex III, as well as glycerol-3-phosphate dehydrogenase of

the electron transport chain are what creates the reactive oxygen species⁵³. Reactive oxygen species are highly reactive and having a large amount of circulating ROS can lead to oxidative stress which is known to cause damage to proteins, DNA, and RNA⁵³. It is hypothesized that these molecules play an integral role in the pathogenesis of cancer, cardiovascular disease, and insulin resistance. Interacting with the ROS, LDL lipids form a variety of lipid oxidation products, which subsequently modifies the apolipoprotein part of the LDL⁹. The most common ROS that have shown to be associated with atherosclerosis are $\cdot\text{O}_2$, OONO^- , H_2O_2 , HO , and O_2^{25} .

The modified LDL apolipoprotein has altered receptor affinity, making it capable to bind with scavenger receptors on macrophages in the walls of the arteries in an uncontrolled manner⁹. Macrophages are cells that play an important role in immune function and are believed to play an important role in atherosclerosis progression²⁶. Once the LDL

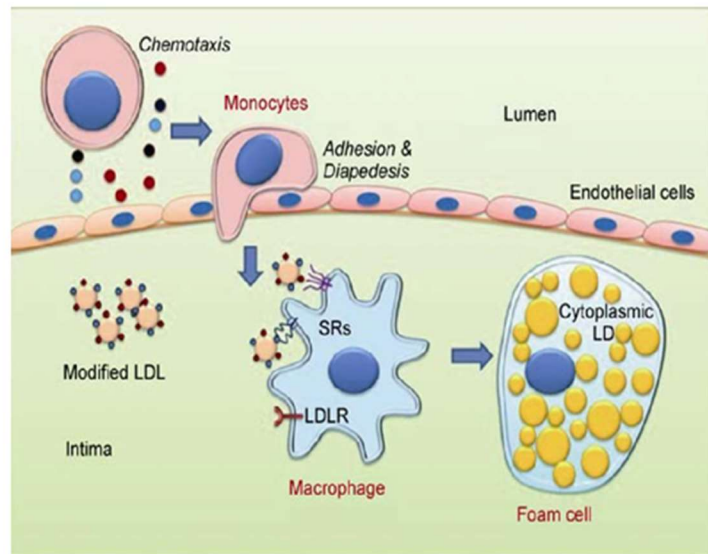


Figure 4. Foam Cell Formation

Yuan, Y., Li, P., & Ye, J. (2012). Lipid homeostasis and the formation of macrophage-derived foam cells in atherosclerosis. *Protein & Cell*, 3, 173-181.



particles are phagocytized into the macrophages, they become foam cells and form fat buildup in the artery wall leading to atherosclerosis⁴. One substance that is used to assess lipid peroxidation levels in vivo are markers of lipid peroxidation called F2-isoprostanes²⁶.

Prostaglandin like compounds also known as F2-isoprostanes are substances that are non-enzymatically formed via lipid peroxidation of arachidonoyl radicals^(13,24). Arachidonic acid is a type of unsaturated fatty acid found in the plasma membrane, where it is bound to phospholipids³⁴.

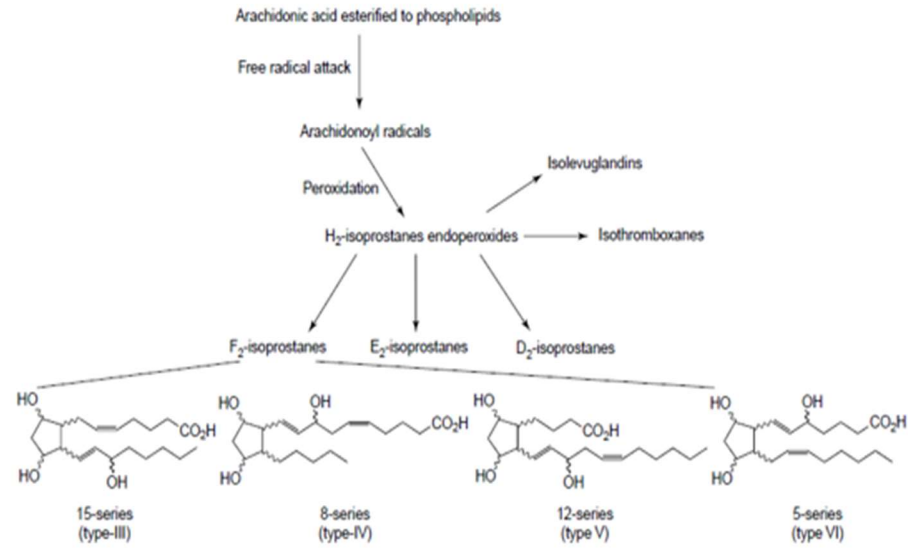


Figure 5. Formation of F2-Isoprostanes from Arachidonic acid.

Cracowski J.L., Durand T., Bessard G. Isoprostanes as a biomarker of lipid peroxidation in humans: Physiology, pharmacology and clinical implications. *Trends Pharmacol. Sci.* 2002;23:360–366. doi: 10.1016/S0165-6147(02)02053-9.

known to be the most efficient measurement of lipid peroxidation in vivo¹³. Elevated levels of F2-isoprostanes are observed in diseases associated with atherosclerosis leading to the hypothesis that there is a strong link between lipid peroxidation and atherosclerosis¹⁴. F2-isoprostanes are easily measured through non-invasive measure such as collections of urine samples. The most common isomer of F2-isoprostanes used in human studies to measure oxidative stress is 8-isoprostaglandin-F₂²³. As with all urinary biomarkers, urinary levels of F2-isoprostanes are standardized by urinary creatinine to account for inter-individual variability in urine diluteness. Unlike ROS which are very reactive and unstable molecule, F2-isoprostanes, their biological “footprints” present chemically stable molecules, which is an important consideration in choosing biomarkers for human studies. For example, 15-series F2-isoprostane concentration remained unchanged in urine samples stored at -20°C¹⁴ and they also remained unchanged when

urine specimens were left at room temperature overnight¹⁴. It is also important to note that urinary F2-isoprostanes levels are not dependent on diet and are not modified by lipid consumption, giving more evidence of its reliability of a good biomarker of in vivo lipid peroxidation¹⁴. F2-isoprostanes as a marker for oxidative stress can also be used as a tool to assess the effects of various antioxidants to see which ones are most effective in achieving homeostasis in oxidative status¹⁵. Antioxidants may help to combat LDL oxidation by protecting cells against ROS-induced damage⁴.

Antioxidant Trials

Oxidative stress is caused by the imbalance of oxidant species and antioxidant species. As mentioned above F2-isoprostanes are used as a marker of oxidative stress and can also be used to assess antioxidants effectiveness¹⁶. Natural antioxidants such as vitamin A, vitamin E, flavonoids, and carotenoids are believed to play an important role in atherosclerosis prevention¹⁶. Although it is believed that an increase in antioxidant intake may reduce the risk of atherosclerosis, by preventing oxidation of LDL, randomized control antioxidant trials showed otherwise^{16,22,23}. Some randomized control trials showed antioxidant supplementation was not associated with decrease in atherosclerosis risk and in some cases may actually be associated with higher increase risk of atherosclerosis events and CVD death^(16,21,22). A meta-analysis by Cherubini et al. showed that the PHS study found that B-Carotene had no effect on CV death or all-cause mortality¹⁶. Three additional studies (PPP, SECURE, and VEAPS) showed that Vitamin E had a neutral effect of the progression of common carotid artery IMT indicating that Vitamin E supplementation was not associated with decrease in atherosclerosis progression. Additionally, the CARET study (the Beta-Carotene and Retinol Efficacy Trial) showed that B-

carotene and Vitamin A increased all-cause mortality, and that these supplements also increased CV death¹⁶. The Cherubini et al. meta-analysis also showed four different random control trials analyzing the association between antioxidants and secondary prevention of atherosclerosis and cardiovascular disease. The studies found no benefits in taking antioxidant supplements¹⁶. The negative and neutral results from these random control trials pose a question of whether increased ROS levels in humans stimulates progression of atherosclerosis

There have been cross-sectional and cohort studies that showed a correlation between increased natural antioxidant intake and decreased atherosclerosis risk^(17,18,19) however, the inverse association in these studies may have been compromised by other chemicals found in foods that contributed to decrease in atherosclerosis risk^{16,18,19}. It is hypothesized that natural antioxidants may only be an effective treatment for those with high oxidative stress levels or those that have a depletion of natural antioxidants^{18,19}. The true relationship between oxidative stress and atherosclerosis is not currently known and further studies are necessary to test the efficacy of antioxidants on CVD prevention before any conclusions or recommendations can be made. In this study we will examine the association of F2-isoprostanes and subclinical atherosclerosis in an attempt to understand the connection between the two.

Research Methods and Procedures

Study Population

This analysis used the Insulin Resistance Atherosclerosis Study (IRAS) to understand the association between oxidative status and subclinical atherosclerosis. The IRAS is a prospective cohort study that was designed to examine the relationships between insulinemia, glycemia, insulin resistance, and cardiovascular risk in a racially and metabolically diverse population including African Americans, non-Hispanic whites, and Hispanics²⁷. Between October 1992 and April 1994, 1625 men and women were recruited to participate in the study and followed up after 5 years²⁷. The participants were between 40-69 years of age at baseline and were recruited from four different clinical centers to represent a range of glucose tolerance (impaired vs normal) and ethnicity²⁷.

Analytic Cohort

Our analytical cohort included participants who were free of diabetes at baseline, underwent both – baseline and follow-up – examinations, and provided urine specimen at baseline (n=901). Among them, several participants were excluded, because of unreliable measurements of urinary F2-isoprostanes. After doing additional analysis on change in carotid intima-media thickness, it was determined that two participants were extreme outliers and were removed leaving a total of 855 participants eligible for the linear and logistic regression analysis.

Outcome of interest: subclinical atherosclerosis

Carotid intima-media artery wall thickness (CIMT) was used as an indicator for subclinical atherosclerosis. CIMT was measured using B-mode ultrasonography at baseline²⁶. The IRAS measured both CCIMT and ICIMT. For this study CCIMT was the primary outcome of interest and ICIMT was the secondary outcome of interest.

Exposure of interest: F2-isoprostanes

Four different urinary F2-isoprostanes were utilized in this study which included iPF (2 alpha)-III, 2,3-dinor-iPF (2 alpha)-III, iPF (2 alpha)-VI, and 8,12-iso-iPF (2 alpha)-VI. Urinary concentration of F2-isoprostane isomers (ng) adjusted for mg urinary creatinine were used as indicators of oxidative status. The adjustment for creatinine is used to account for inter-individual variability in urine diluteness. The isomers were measured in samples of morning spot urine specimens at baseline. The specimens were stored at -70° C and quantified using liquid chromatography-tandem mass spectrometry²⁸. Creatinine was assayed using a fast electrospray ionization-tandem mass spectrometry method²⁸.

The F2-isoprostane index is a variable that was previously created as a composite measurement of all four F2-Isoprostane isomers³⁰. The index was created using the formula $[(X1i - M1)/SD1 + (X2i - M2)/SD2 + (X3i - M3)/SD3 + (X4i - M4)/SD4]/4$, where “*i*” is a notation for a participant³⁰. The values of four F2-isoprostanes species (X1–4) were standardized by subtracting estimated mean (M1–4) and divided by standard deviation (SD1–4)³⁰. Previous studies have shown that the F2-isoprostane index is a good summary mean of the four F2-isoprostane isomers^{29,30}.

Covariates

Demographic factors included baseline age, gender, and ethnicity. Lifestyle risk factors for CVD included self-reported baseline smoking status (ever/never), and Body Mass Index (BMI), as weight in kilograms divided by height in meters squared. Glucose tolerance status was determined through a 75 g oral glucose tolerance test at baseline and at follow-up and classified according to the World Health Organization criteria⁶⁰. Plasma lipoprotein measurements were obtained from fasting, single, fresh plasma samples by using Lipid Research Clinics methods⁶⁰. To measure HDL levels, precipitation of apo B-containing lipoproteins with MnCl₂ and heparin was utilized and the cholesterol content in the supernatant was measured in a separate autoanalyzer⁶⁰. LDL cholesterol was calculated as the difference between the HDL cholesterol and the cholesterol from the bottom fraction of VLDL particles after it was isolated via ultracentrifugation⁶⁰. Triglycerides were measured enzymatically after correction for free glycerol⁶⁰.

History of CVD events at baseline was defined as a history or presence of at least one of the following conditions: self-reported stroke, angina, heart problems, myocardial infarction, or hypertension. Hypertension was determined by blood pressure measurements using standard mercury column sphygmomanometer. Three measurements were taken during the examinations at baseline and follow-up and the second and third measurement was used to determine hypertension²⁹. Hypertension was defined as a systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than 90 mm Hg, and/or a current regimen of antihypertensive medications²⁹

Statistical analysis

All analysis was performed using SAS 9.3. For model building bivariate analyses examining the association between outcome or main predictor (Common and Internal CIMT/ F2-isoprostane isomers) and categorical covariates were performed using the Wilcoxon and Kruskal-Wallis tests to test for possible confounders. Bivariate analyses examining the association between outcome or main predictor (Common and Internal CIMT/ F2-isoprostane isomers) and continuous covariates were performed using the Spearman correlation coefficient to test for possible confounders. To determine whether elevated levels of oxidative status can predict progress in subclinical atherosclerosis, we first dichotomized change in CCIMT and ICIMT into two categories. Taking into account measurement error of IMT based on previous studies, which is between 0.08 mm and 0.20 mm⁵⁵, we considered > 15% increase in CIMT during the 5-year follow-up as the indicator of progression in subclinical atherosclerosis. No change or decrease in CIMT during the observation period was considered as lack of progression in subclinical atherosclerosis. Those that had increased CIMT were considered cases, whereas those with no CIMT increase were considered non-cases. A total of 178 participants were classified as cases with clear indication of subclinical atherosclerosis using CCIMT and 243 as cases using ICIMT.

Univariate logistic regression examined the crude relationship between CIMT and the F2-isoprostane isomers. Multivariate logistic regression examined the relationship between CIMT and F2-isoprostane isomers after adjustment for age, sex, ethnicity, IGT status, and BMI. To verify the result from the logistic regression, we analyzed changes in CCIMT and ICIMT as continuous variables using linear regression. Natural log transformation of the CIMT data was

utilized to address skewness in the data. The outcome was modeled as follow-up log transformed CIMT, the main exposure was the F2-isoprostane index, and the predictors included baseline CIMT (log transformed), and the following covariates: age, sex, ethnicity, smoking status, BMI, and systolic blood pressure.

A Sensitivity analysis was performed to address the influence of cardiovascular disease history as a possible intermediate variable. Those that had history of CVD were excluded from the analysis and additional logistic regression analyses were performed. At baseline 403 individuals were considered to have a history of CVD leaving a total of 454 participants eligible for the sensitivity analysis.

Results

Baseline Descriptive Characteristics

In this study population with 857 metabolically and ethnically diverse participants, the average age was 54.56 and there were more females (57.64 %) than males (42.36). There were slightly more non-Hispanic whites (40.02%) in the study population, compared to Hispanics (32.21%) and non-Hispanic blacks (27.77%) which can be seen in Table 1. About one-third of the study population had impaired glucose tolerance (32.44%) and about half had a history of CVD events or hypertension (47.03%) at baseline (table 1).

Table 1. Descriptive Statistics for Baseline Characteristics

Variable	Level	N (%) N= 857
Ethnicity	Non-Hispanic Black	238 (27.77)
	Hispanic	276 (32.21)
	Non-Hispanic White	343 (40.02)
Sex	Male	363 (42.36)
	Female	494 (57.64)
IGT-Status	Normal	579 (67.56)
	IGT	278 (32.44)
	Diabetes	0
CVD Events	Yes	403 (47.03)
	No	454 (52.97)
Smoking Status	Never	398 (46.44)
	Past/Current	459 (53.56)
Age (years)	Mean	54.56
	Median	54.00
	Std Dev	8.31
	IQR	14
BMI (kg/m ²)	Mean	28.46
	Median	27.29
	Std Dev	5.66
	IQR	5.75

Baseline Characteristics for Exposure and Outcomes of Interest

The main outcome of interest, common carotid artery intima-media thickness (CCIMT), and the secondary outcome of interest, internal carotid artery intima-media thickness (ICIMT), differed slightly at baseline and follow up. Mean ICIMT was measured to be greater than mean CCIMT at baseline (0.91 mm vs 0.80 mm) and at follow up (1.01 mm vs 0.84mm) respectively (Table 2). The mean levels of F2-isoprostane in the study population varied among the four isomers ranging from 0.25 ng/mg creatinine (CN) up to 6.48 ng/mg creatinine (CN) as shown in Table 2.

Table 2. Descriptive Statistics for Outcomes (CCIMT, ICIMT) and Predictors (F2-Isoprostanes)

Variables (Carotid Artery IMT)	Mean (SD) N=857	Median (IQR) N=857
Common Carotid Artery IMT-Baseline (mm)	0.80 (0.22)	0.76 (0.20)
Common Carotid Artery IMT-Follow-up (mm)	0.84 (0.25)	0.78 (0.22)
Internal Carotid Artery IMT-Baseline (mm)	0.91 (0.40)	0.79 (0.25)
Internal Carotid Artery IMT-Follow-up (mm)	1.01 (0.55)	0.82 (0.33)
Variables (F2-Isoprostanes)	Mean (SD)	Median (IQR)
2,3-dinor-iPF(2a)-III, ng/mg CN	4.35 (3.00)	3.71 (2.74)
iPF(2a)- III, ng/mg CN	0.25 (0.19)	0.20 (0.17)
iPF(2a)- IV, ng/mg CN	6.48 (4.16)	5.37 (4.18)
8,12-iso-iPF(2a)-IV, ng/mg CN	4.15 (2.87)	3.46 (2.79)
F2-isoprostane Index, ng/mg CN	-0.06 (0.82)	-0.23 (0.83)

Confounder Analysis

To identify potential confounders, we selected variables that are known to be associated with subclinical atherosclerosis and/or oxidative status and examined their crude association with the outcomes (Table 3) and primary predictor (Table 4). The variables that were associated with CCIMT/ ICIMT included sex, ethnicity, smoking status, and IGT status. Males had higher CCIMT/ ICIMT than females, non-Hispanic blacks had higher CCIMT/ ICIMT than both non-Hispanic whites and Hispanics. Those with IGT had greater CCIMT/ ICIMT compared to those with normal glucose tolerance (Table 3) at baseline. For IGT status at follow-up, those that were diagnosed with diabetes had greater CCIMT/ICIMT compared to those with normal glucose tolerance and those with IGT. Additionally, those that currently smoke or used to smoke had higher CCIMT/ ICIMT levels than those that never smoked. Based on the Spearman correlation age, BMI, LDL, and systolic blood pressure are associated with CCIMT/ ICIMT progression and has a direct correlation with CCIMT/ ICIMT indicating that as age, BMI, LDL, and systolic blood pressure increases so does CCIMT/ ICIMT levels (Table 3).

Examining crude associations between the F2-isoprostane index and suspected confounders in Table 4, we found that sex, ethnicity, smoking status, and IGT status at follow-up were associated with baseline oxidative status. Females, Hispanic, those with a history of smoking, and those with impaired glucose tolerance had higher levels of systemic F2-isoprotanes (Table 4). BMI and HDL were associated with and directly correlated with oxidative status. Age was associated with and inversely correlated with F2-isoprostanes.

Table 3. Association between Outcomes, CCIMT and ICIMT, and Characteristics of Interest

Variables	CCIMT Baseline, mm Median (IQR)	CCIMT follow-up, mm Median (IQR)	ICIMT Baseline, mm Median (IQR)	ICIMT follow-up, mm Median (IQR)
Sex				
Male	0.80 (0.23)	0.83 (0.26)	0.82 (0.29)	0.87 (0.44)
Female	0.74 (0.20)	0.76 (0.18)	0.76 (0.22)	0.79 (0.27)
P-value*	<.0001	<.0001	<.0001	<.0001
Ethnicity				
Black	0.81 (0.24)	0.83 (0.24)	0.83 (0.22)	0.85 (0.30)
Hispanic	0.73 (0.18)	0.73 (0.19)	0.74 (0.18)	0.77 (0.26)
White	0.76 (0.19)	0.80 (0.22)	0.79 (0.34)	0.84 (0.43)
P-value*	<.0001	<.0001	<.0001	<.0001
CVD Events (Baseline)				
Yes				
No	0.82 (0.23)	0.83 (0.25)	0.80 (0.31)	0.85 (0.43)
P-value*	0.73 (0.16) <.0001	0.75 (0.20) <.0001	0.77 (0.22) 0.012	0.80 (0.25) <.0001
IGT-Status				
Normal	0.75 (0.22)	0.76 (0.21)	0.78 (0.24)	0.80 (0.30)
IGT	0.80 (0.20)	0.80 (0.25)	0.79 (0.28)	0.83 (0.37)
Diabetes	N/A	0.84 (0.28)	N/A	0.88 (0.42)
P-value*	0.0005	<.0001	0.15	0.032
Smoking Status				
Never	0.74 (0.21)	0.76 (0.19)	0.77 (0.23)	0.80 (0.24)
Past/Current	0.78 (0.21)	0.80 (0.26)	0.79 (0.31)	0.85 (0.46)
P-value*	0.0082	0.0008	0.0052	0.0002
LDL				
Spearman correlation	0.15	0.084	0.18	0.05
P-Value	<.0001	0.0158	<.0001	0.2005
HDL				
Spearman correlation	0.0021	-0.070	-0.049	-0.061
P-Value	0.9531	0.048	0.18	0.083
Age (baseline years)				
Spearman correlation	0.37	0.36	0.18	0.22
P-Value	<.0001	<.0001	<.0001	<.0001

BMI (kg/m ²)				
Spearman correlation	0.14	0.17	0.06	0.0015
P-Value	0.0001	<.0001	0.11	0.97
Systolic Blood Pressure				
Spearman correlation	0.42	0.39	0.12	0.16
p-value	<.0001	<.0001	0.0010	<.0001

*Wilcoxon and Kruskal-Wallis test performed

Table 4. Association between Baseline F2-isoprostanes and Characteristics of Interest

Variables	2,3-dinor- iPF(2a)-III	iPF(2a)- III	iPF(2a)- IV	8,12-iso- iPF(2a)-IV	F2-isoprostane Index
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Sex					
Male	2.95 (1.84)	0.16 (0.12)	4.22 (2.79)	3.20 (2.13)	-0.43 (0.53)
Female	4.47 (3.23)	0.24 (0.20)	6.39 (4.76)	3.62 (3.33)	0.03 (1.00)
P-value*	<.0001	<.0001	<.0001	0.0038	<.0001
Ethnicity					
Black	3.23 (2.29)	0.16 (0.14)	4.34 (2.86)	2.90 (2.08)	-0.43 (0.60)
Hispanic	4.46 (3.15)	0.25 (0.20)	6.91 (4.68)	4.03 (3.64)	0.08 (0.93)
White	3.56 (2.70)	0.20 (0.16)	5.37 (3.96)	3.48 (2.35)	-0.25 (0.78)
P-value*	<.0001	<.0001	<.0001	<.0001	<.0001
CVD Events (baseline)					
Yes	3.78 (2.63)	0.19 (0.17)	5.08 (3.66)	3.35 (2.69)	-0.28 (0.83)
No	3.69 (2.81)	0.21 (0.17)	5.78 (4.62)	3.53 (2.88)	-0.13 (0.85)
P-value*	0.48	0.10	0.0017	0.063	0.066
IGT-Status (baseline)					
Normal	3.67 (2.55)	0.20 (0.18)	5.39 (3.98)	3.32 (2.68)	-0.23 (0.78)
IGT	4.08 (3.09)	0.20 (0.16)	5.29 (5.17)	3.56 (2.95)	-0.20 (0.93)
Diabetes	N/A	N/A	N/A	N/A	N/A
P-value*	0.022	0.54	0.93	0.21	0.28
Smoking Status (baseline)					
Never	3.70 (2.91)	0.20 (0.17)	5.49 (4.68)	3.52 (2.93)	-0.18 (0.80)
Past/Current	3.74 (2.61)	0.21 (0.17)	5.28 (4.00)	3.40 (2.64)	-0.23 (0.83)
P-value*	0.041	<.0001	0.0105	0.12	0.0003

LDL (baseline) Spearman correlation P-Value	-0.072 0.039	-0.096 0.0055	-0.044 0.20	-0.024 0.49	-0.067 0.054
HDL (baseline) Spearman correlation P-Value	0.11 0.0013	0.13 0.0002	0.17 <.0001	0.018 0.60	0.13 0.0001
Age (baseline) Spearman correlation P-Value	-0.031 0.35	-0.010 0.76	-0.033 0.34	-0.17 <.0001	-0.072 0.036
BMI (kg/m ²) (baseline) Spearman correlation P-Value	0.18 <.0001	0.0043 0.89	0.045 0.18	0.10 <.0024	0.10 0.0034
Systolic Blood Pressure (baseline) Spearman correlation p-value	0.030 0.39	-0.035 0.30	-0.080 0.023	-0.038 0.27	-0.031 0.36

*Wilcoxon and Kruskal-Wallis test performed

After considering the results in Tables 3 and 4, six of the total ten potential covariates were selected for the final linear and logistic regression analyses. The final model included sex, ethnicity, age, BMI, smoking status, and systolic blood pressure as confounders.

Univariate and Multivariate Logistic Regression Analysis

Logistic regression was used to estimate the association between oxidative status and progression in subclinical atherosclerosis. We found inverse association between baseline F2-isoprostane levels and CCIMT progression (OR = 0.77, 95% CI [0.61-0.98]) before being adjusted for other covariates. This crude association indicated that the exposure, elevated F2-isoprostanes in urine, are more likely to be found among non-cases (Table 5). After adjustment for the selected covariates F2-isoprostanes continue to have an inverse association with CCIMT (OR= 0.76 ,95% [0.58-1.01]). F2-isoprostanes were not associated with ICIMT before

adjustment (OR = 1.01, 95% CI [0.83-1.20]) or after adjustment (OR = 0.94 95%, CI [0.76-1.17]).

Table 5. Logistic Regression Analysis between Outcomes of Interest and Primary Predictor

	Unadjusted Odds Ratio (95% CI)		Adjusted Odds Ratio** (95% CI)	
	Common CIMT*	Internal CIMT*	Common CIMT*	Internal CIMT*
2,3-dinor-iPF(2a)-III]	0.97 (0.91-1.03)	1.01 (0.96-1.06)	0.97 (0.90-1.03)	1.01 (0.92-1.07)
iPF(2a)- III	0.33 (0.12-0.95)	1.07 (0.49-2.31)	0.32 (0.11-0.97)	0.95 (0.41-2.21)
iPF(2a)- IV	0.97 (0.93-1.01)	0.98 (0.94-1.02)	0.96 (0.92-1.01)	0.97 (0.93-1.02)
8,12-iso-iPF(2a)-IV	0.91 (0.85-0.98)	1.00 (0.94-1.05)	0.91 (0.84-0.98)	1.01 (0.92-1.07)
F2-isoprostane Index	0.77 (0.61-0.98)	1.01 (0.83-1.20)	0.76 (0.58-1.01)	0.94(0.76-1.17)

*Odds ratio (95% CI)

** Odds ratio adjusted for age, sex, ethnicity, BMI, smoking status, and systolic blood pressure

Univariate and Multivariate Linear Regression Analysis

After natural log transformation of the CCIMT/ICIMT variables the linear regression models showed a tendency for inverse association between urinary F2-isoprostanes and CCIMT/ICIMT assessing both adjusted and unadjusted parameter estimates (Table 6). The logistic and linear models are compatible with each other and indicate that elevated F2-isoprostanes levels do not predict progress in CCIMT/ICIMT progression.

Table 6. Linear Regression Analysis between Outcomes of Interest and Primary Predictor

	Beta Coefficient estimates for F2-isoprostane Index (95% CI)	Adjusted Beta Coefficient estimates for F2-isoprostane Index (95% CI) *
Common CIMT	-0.014(- 0.028 to -0.00028)	-0.014(-0.029 to 0.00062)
Internal CIMT	-0.018(-0.045 to 0.0083)	-0.0021 (-0.030 to 0.026)

*Adjusted for age, sex, ethnicity, BMI, smoking status, and systolic blood pressure

Cardiovascular Disease Sensitivity Analysis

CVD events were not placed in the adjusted regression analyses due to the fact that CVD events are not considered a confounding variable. We cannot use CVD history as a confounder, because this is an intermediate variable. We can only do a sensitivity analysis to see how our association changes when all previous CVD morbidity is excluded. As Table 1 shows, there are 403 individuals that had a history of CVD at baseline. Once these participants were excluded, we ran a logistic regression sensitivity analysis with the remaining 454 participants as seen in Table 7. We saw no association between subclinical atherosclerosis progression and F2-isoprostanes for CCIMT (OR 0.78, 95% CI [0.53-1.15]) and continued to see no association with ICIMT (OR 0.98, 95% CI [0.72-1.34]) after adjustment. After examining Tables 5-7 we concluded that these findings indicate that elevated levels of urinary F2-isoprostanes do not predict subclinical atherosclerosis progression when examining both common and internal carotid artery intima-media thickness.

Table 7. Logistic Regression Sensitivity Analysis: Excluding those with Baseline CVD Morbidity

	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
	F2-Isoprostane Index	
Common CIMT	0.76 (0.54-1.06)	0.78 (0.53-1.15)
Internal CIMT	1.10 (0.84-1.42)	0.98 (0.72-1.34)

*Adjusted for age, sex, ethnicity, BMI, smoking status, and systolic blood pressure

Discussion

The present study examined The Insulin Resistance Atherosclerosis Study which conducted research on an ethnically and metabolically diverse study population aged 40-69 with the goal to understand the association between insulin resistance, cardiovascular disease and its risk factors. The diversity makes it generalizable to the US population aged 40-69 and the vast data collection of risk factors makes it a suitable study for additional analysis.

Atherosclerosis is a progressive disease that is responsible for the onset of numerous cardiovascular diseases such as stroke, cardiac arrest, and angina. Atherosclerotic cardiovascular disease is the leading cause of death worldwide and its burden is expected to continue to rise over the next decades, especially in low- and middle-income regions³⁶. Previous studies have demonstrated that there are racial disparities in cardiovascular disease mortality and morbidity^{36,37,37}. Studies have shown that non-Hispanic blacks have higher risk of cardiovascular disease mortality and morbidity^{36,37,37}. Non-Hispanic blacks are 2 to 3 times more likely to die from stroke or heart disease³⁶. The present study also found racial disparities that aligned with

previous studies. Non-Hispanic blacks had the highest level of CCIMT and ICIMT among the three groups. At follow up non-Hispanic blacks had a median CCIMT measurement of 0.83 mm, compared to non-Hispanic whites (0.80 mm) and Hispanics (0.73). The same disparity was seen in ICIMT between non-Hispanic blacks, Hispanics, and non-Hispanic whites (0.85 mm vs 0.77 mm vs 0.84 mm) respectively.

Previous studies have also determined that there are disparities in sex for cardiovascular disease^{39,40}. There is a 10-year difference between first cardiovascular disease event in women compared to men³⁹. Cardiovascular event rates are low in women that are pre-menopausal, and it is believed that exposure to endogenous estrogens during earlier stages of life delays the manifestation of atherosclerotic disease in women^{39,40}. The current study also saw sex disparities in subclinical atherosclerosis between men and women. There was significant difference between men and women at follow-up for both median CCIMT (men: 0.83 mm vs women:0.76 mm, $p<.0001$) and median ICIMT (men:0.87 mm vs women:0.79 mm, $p<.0001$).

One major risk factor for atherosclerosis is diabetes. Adults with diabetes are two to four times more likely to die from heart disease than adults without diabetes⁴². About 68% of people 65 years of age are older who have diabetes die from heart disease and 16% die from stroke⁴². It is believed that this association between diabetes and cardiovascular disease has to do with the role hyperglycemia plays in the pathogenesis of atherosclerosis^{41,42,44}. The present analysis using IRAS data also saw a significant difference ($p<0.0001$) through an impaired glucose tolerance gradient. At follow-up those with normal glucose tolerance had the lowest median CCIMT (0.76 mm) followed by those with impaired glucose tolerance (0.80 mm), and diabetes (0.84 mm). The same gradient between the three groups was seen in ICIMT as well (0.80 mm vs 0.83 mm vs 0.88 mm, $p<0.001$).

Although there have been studies indicating that reactive oxygen species play an important role in the pathology of many chronic diseases including cardiovascular disease^{14,33,58}, based on results from this study, the typical way of thinking about the relationship between F2-isoprostanes and cardiovascular disease may not be completely accurate. Reactive oxygen species are believed to play an important role in the pathology of cardiovascular disease progression however previous randomized clinical trials of antioxidants failed to conclude any beneficial effects of antioxidant intake on cardiovascular disease morbidity or mortality^{22,23}. The present study examined urinary F2-isoprostanes, a reliable measure of ROS and oxidative status, and did not see a direct association between subclinical atherosclerosis progression and increased levels of systemic F2-isoprostanes when examining biomarkers for subclinical atherosclerosis CCIMT (OR = 0.76) and ICIMT (OR = 0.94) after adjustment. Based on this study, it seems that high level of F2-isoprostanes are not associated with progression in subclinical atherosclerosis. The inverse association between CCIMT progression and F2-isoprostane levels is the opposite of what is expected based on previous studies^{33,58}.

Although direct correlation of F2-isoprostanes with cardiovascular disease is the more traditional way of thinking, another biological hypothesis about F2-isoprostanes that would align with the results shown in this study is that F2-isoprostanes are possible markers for fatty acid oxidation, mitochondrial metabolism, in addition to markers for reactive oxygen species levels. There have been studies on conditions that have to do with intense fat oxidation such as fasting and exercise that showed a direct correlation with increase F2-isoprostanes⁴⁵. Steensberg, et al. noted that F2-isoprostanes increased approximately 1.6-fold in response to moderate exercise⁴⁴. Additionally, Il'yasova, et al. showed a direct correlation between C2 and C12, which are metabolic markers of fatty acid oxidation and F2-isoprostanes, adjusted beta coefficients are

0.109 and 0.072 respectively, giving more evidence to the theory of F2-isoprostanes being markers of mitochondrial fatty acid oxidation²⁹.

As table 4 shows ethnicity is associated with F2-isoprostanes with non-Hispanic blacks having the lowest F2-isoprostane index (-0.43ng/mg creatinine) followed by non-Hispanic whites and Hispanics (-0.25 ng/mg creatinine, 0.008 ng/mg creatinine) respectively. These findings align with previous findings that showed non-Hispanic blacks, who are known to have slower mitochondrial oxidation as well as lower levels of fat oxidation, also had lower levels of systemic F2-isoprostanes compared to non-Hispanic whites³⁴ further suggesting that F2-isoprostanes are possible markers of positive mitochondrial activity and not necessarily just markers of harmful oxidative damage.

F2-isoprostanes are markers of free radical oxidative stress and although free radical oxidative stress may not be associated with cardiovascular disease risk, it does not mean that the idea of oxidative stress being an important part of cardiovascular disease pathology is wrong. Recent evidence has shown that non-free radical marker of oxidative stress; plasma aminothiols, high cystine levels, and low glutathione, were associated with coronary heart disease⁵⁶. Additionally, a study by Dhawan, et al. presented evidence that lower plasma glutathione levels ($r=0.39$, $p=0.01$) as well as higher cystine/glutathione ratio ($r=-0.29$, $p=0.04$) were independent predictors of impaired coronary microvascular function which can lead to higher susceptibility to atherosclerosis⁵⁶. Plasma aminothiols such as cysteine, cysteinyl glycine, and glutathione are considered good non-free radical markers for oxidative stress⁵⁸. All of these species interreact through the thiol redox status which plays an important role in redox homeostasis. Redox homeostasis is an important condition for normal cellular function⁵⁸. When excessive amounts of total plasma homocysteine and cysteine are produced or when low levels of glutathione are

produced it can result in pro-oxidant effects⁵⁸. Some known effects of redox homeostasis imbalance include endothelial dysfunction as well as LDL oxidation which can result in the onset of atherosclerosis⁵⁸. Evidence has shown that both high plasma homocysteine and cysteine levels as well as low glutathione levels are well known conditions associated with atherosclerosis and CVD⁵⁸⁻⁶⁰.

It could be hypothesized that free radical oxidative stress may be related to mitochondria activity and fatty acid oxidation, while non-free radical oxidative stress is more relevant to the development of atherosclerosis and cardiovascular disease. Therefore, it would be expected that F2-isoprostanes would show no signs of association with atherosclerosis and would possibly be a marker of decrease risk if F2-isoprostanes were markers of fatty acid oxidation. Non-free radical oxidative stress markers, such as plasma thiols, may be a more important factor in atherosclerosis progression and CVD events. Future studies are needed to get a better understanding of the different forms of oxidative markers and their influence on different diseases.

Conclusion

The result from this study indicate that urinary F2-isoprostanes do not predict progress in subclinical atherosclerosis when examining CCIMT (OR 0.76, 95% CI 0.58-1.01) and ICIMT (OR 0.94, 95% CI 0.76-1.17). The CVD sensitivity analysis performed also indicated that F2-isoprostanes do not predict progress in subclinical atherosclerosis when examining CCIMT (OR 0.78, 95% CI 0.53-1.15) and ICIMT (OR 0.98, 95% CI 0.72-1.34) after excluding participants with previous CVD history. Although these finding contradict with the typical way of viewing the relationship between F2-isoprostanes and atherosclerosis, the findings may align with the theory that F2-isoprostanes levels, reactive oxygen species levels, and mitochondrial fatty acid oxidation are intertwined. More research should be dedicated to understanding the role free radical oxidative markers and non-free radical oxidative markers play in the pathology of cardiovascular disease so a conclusive understanding of the relationship between oxidative status and CVD can be attained. Additionally, research focused on F2-isoprostanes as makers of mitochondrial fatty acid oxidation is needed to gain a better understanding of this hypothesis. Due to the severity of CVD mortality and morbidity in so many countries, it is vital to understand the important pathological steps of its main cause, atherosclerosis formation, in an effort to create early detection methods that can serve as primary prevention measures for cardiovascular events.

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