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Plasma F_2-Isoprostanes and Coronary Artery Calcification: The CARDIA Study

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Background: **Oxidation of lipids in lipoproteins and cells may initiate and enhance the early development of cardiovascular disease.**

Method and Results: **We assayed F_2-isoprostanes, oxidation products of arachidonic acid, by gas chromatography–mass spectrometry in a biracial cohort of 2850 young healthy adult men and women. Coronary artery calcification (CAC), a component of coronary artery atherosclerosis, was detectable in 10% of the cohort and appeared to be in its initial stages (Agatston scores <20 in 47% and <100 in 83% of CAC-positive participants). After adjusting for sex, clinical site, age, and race, the presence of any CAC was 24% more likely among those with high vs low concentrations of F_2-isoprostanes [odds ratio (OR) = 1.24 per 92.2 pmol/L (32.7 ng/L; 1 SD of F_2-isoprostanes); 95% confidence interval (CI), 1.09–1.41]. The OR was only slightly attenuated [1.18 per 92.2 pmol/L (32.7 ng/L); CI, 1.02–1.38] after further adjustment for body mass index, smoking, serum lipids, C-reactive protein, antioxidant supplementation use, diabetes, and blood pressure. As a continuous variable, the Agatston score increased by 6.9% per 92.2 pmol/L (32.7 ng/L) of F_2-isoprostane concentration (P <0.01). Whereas CAC prevalence was lower in women than men, mean (SD), F_2-isoprostanes were higher in women {190 (108.9) pmol/L [67.4 (38.6) ng/L]} than in men {140.4 (55.6) pmol/L [49.8 (19.7) ng/L]}. Nevertheless, F_2-isoprostanes were associated with an increased risk of CAC in both sexes.**

Conclusion: **This association between increased concentrations of circulating F_2-isoprostanes and CAC in young healthy adults supports the hypothesis that oxidative damage is involved in the early development of atherosclerosis.**

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The pathogenesis of atherosclerosis may involve the oxidation of LDL particles and their uptake by macrophages as well as production of reactive oxygen species and localized oxidative damage of cells and LDL *(1)*. This oxidation of LDL particles can initiate the formation of foam cells and promote further oxidative damage, inflammation, chemotactic activity, cytotoxicity, and endothelial dysfunction *(2)*. These activities may lead to the formation of fatty streaks and, ultimately, induce the formation of atherosclerotic plaques and vessel stenosis.

Epidemiologic evidence also supports this hypothetical model. In cross-sectional studies, individuals with established cardiovascular disease had higher concentrations of lipid oxidation products than controls *(3)*, and in prospective studies, autoantibodies to oxidized LDL *(4)* preceded the later development of cardiovascular disease. Numerous observational studies have found an inverse association between the intake of diets high in antioxidants and the risk of cardiovascular disease *(5, 6)*, but clinical trials with pharmacologic doses of antioxidant vitamins, a treatment that might combat oxidative stress, have generally been unsuccessful in the prevention of cardiovascular disease *(7, 8)*. Direct assessment of oxidative damage has occurred only in patients at advanced stages of atherosclerosis *(9)*, when oxidative damage could be induced by the disease process or may not cause plaque development. No studies have measured oxidative damage in plasma during the early stages of atherosclerosis in humans, indicating a need to reexamine its potentially etiologic role in atherosclerosis.

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This work was conducted as part of the YALTA Study, an ancillary study of the CARDIA cohort, and as part of the CARDIA Study, including all clinical sites of the CARDIA Study.

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Plasma F_2-isoprostanes, a new measure of oxidative damage *(10)*, are formed in vivo essentially exclusively from the reaction of free radicals with arachidonic acid *(11)*; trace amounts of F_2-isoprostanes may, in theory, be formed by cyclooxygenase-catalyzed reactions, but they are insignificant contributors. Plasma concentrations of F_2-isoprostanes are modulated by common oxidative stresses *(12, 13)*. We hypothesized that plasma F_2-isoprostanes are associated with early atherosclerosis as measured by coronary artery calcification (CAC)[6] in a population-based study of healthy young adults.

Participants and Methods

PARTICIPANTS

This cross-sectional study was part of the Young Adult Longitudinal Study of Antioxidants (YALTA), an ancillary study to Coronary Artery Risk Development in Young Adults (CARDIA), which tracks the evolution of cardiovascular disease risk factors in humans who were 18–30 years of age in 1985–1986. Recruitment of 5115 black and white men and women was population based in Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA *(14, 15)*, with participants reexamined at years 2, 5, 7, 10, and 15 *(16)*. Most individuals (n = 3672) participated in the year 15 examination that occurred between May 2000 and June 2001, with this analysis including participants who had available fasting plasma samples and CAC measured by computed tomography (CT; n = 2850). Of the 3672 individuals, CT was completed in 78% of black females, 81% of black males, 83% of white females, and 89% of white males and in 78% of current smokers vs 84% of nonsmokers. Individuals who had CAC measurements were slightly (0.6 years) older and had 0.3 years more education, 1.4 mg/L lower C-reactive protein (CRP; geometric mean), and 1 kg/m^2 lower body mass index (BMI) than those who did not. Individuals with any possible impairment of kidney function (serum creatinine concentrations >12 mg/L for women and 15 mg/L for men at years 0, 10, or 15) were excluded from the sample. Of the sample population (n = 2850), 84 had diabetes (55 receiving treatment) and 63 used lipid-lowering drugs.

DATA COLLECTION

Demographic, smoking, and drug treatment information was collected, and height and weight were measured to calculate BMI (kg/m^2). Resting systolic and diastolic blood pressure were measured using the means of the second and third random zero sphygmomanometer measurements *(17)*.

BIOCHEMICAL MEASUREMENTS

After an overnight fast, blood samples were collected in EDTA-containing and serum Vacutainer Tubes. Blood in the serum Vacutainer Tubes was allowed to clot at room temperature, and the serum was processed and frozen within 90 min of collection. After collection, the blood samples in EDTA-containing Vacutainer Tubes were mixed and immediately placed at 4 °C. Within 90 min, plasma was separated by centrifugation, transferred to airtight vials, flash-frozen on dry ice, and stored at −70 °C. Subsequently, serum and plasma samples were shipped by overnight delivery on dry ice to the respective laboratories and stored at −70 °C until analysis. In separate studies, we evaluated the stability of F_2-isoprostanes under various processing procedures (including immediate processing and storage or holding of whole blood for various times and under various temperatures followed by processing and storage). These studies have shown that the F_2-isoprostanes were completely stable under the collection and processing procedures used in this study (manuscript in preparation). No plasma F_2-isoprostanes were formed or lost during these procedures. Consequently, no F_2-isoprostanes were formed ex vivo, including by cyclooxygenase, in our samples.

Plasma total cholesterol, HDL-cholesterol (HDL-C), and triglycerides were measured enzymatically within 6 weeks of collection *(18)* at the Northwest Lipid Research Laboratory at the University of Washington (Seattle, WA). HDL-C was determined after precipitation of LDL-containing lipoproteins with dextran sulfate/magnesium chloride *(19)*. LDL-cholesterol (LDL-C) was calculated by use of the Friedewald equation *(20)*; the few individuals with triglycerides >4.52 mmol/L (4000 mg/L) were excluded from this calculation. High-sensitivity enzyme-linked immunosorbent assays measured serum CRP at the Department of Pathology, University of Vermont, as described previously *(21)*. The test-retest correlation, in 448 blind duplicate samples, was 0.98–0.99 for total cholesterol, HDL-C, LDL-C, triglycerides, and CRP. Serum α-tocopherol was measured as described previously and had a test-retest correlation of 0.93 *(22)*.

Plasma free F_2-isoprostanes were measured with a gas chromatography–mass spectrometry-based method *(23)* by the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota (Minneapolis, MN). The method used an internal standard, $[^2H_4]$8-iso-$PGF_{2\alpha}$ (>98% pure; Caymen Chemical), wherein the deuterium atoms were located at the nonexchangeable positions 3 and 4 of the molecule. The internal standard was added directly to the plasma samples before the initiation of analysis and therefore accounted for recovery of F_2-isoprostanes. The assay provides a quantitative measurement of plasma F_2-isoprostanes; the specificity of the assay for F_2-isoprostanes has been demonstrated previously *(23)*. Analytical variation of the method was 10% for each of three control pools, which had a range of concentrations. In 368 blind duplicate pairs

[6] Nonstandard abbreviations: CAC, coronary artery calcification; YALTA; Young Adult Longitudinal Study of Antioxidants; CARDIA, Coronary Artery Risk Development in Young Adults; CT, computed tomography; CRP, C-reactive protein; BMI, body mass index; HDL-C and LDL-C, HDL- and LDL-cholesterol, respectively; and CI, confidence interval.

(which included variance attributable to sample handling, labeling, and analysis), the analysis had an overall technical error and test-retest correlation of 20.1% and 0.84, respectively. In population studies, these measures were always larger than the analytical variation and were typical for this type of analysis in a large epidemiologic study. In samples from non-CARDIA participants, assayed by the same methods, the intraclass correlation was 0.84 for five samples drawn at weekly intervals in 14 people, indicating that the within-subject variation was small and not much greater than the laboratory variation (10%); between-subject variation accounted for most of the variation (84%) in F_2-isoprostane concentrations. All samples were analyzed within 1 year of collection. Long-term studies at the MEBRL of three control pools indicated that plasma concentrations of F_2-isoprostanes were stable for >16 months at −70 °C.

CAC MEASUREMENTS

CAC was measured in Oakland, CA and Chicago, IL by electron beam CT (Imatron, Inc.) and in Birmingham, AL and Minneapolis, MN by multidetector CT (General Electric Lightspeed in Birmingham and Siemens S4+ Volume Zoom in Minneapolis). The CT scanning protocol included a hydroxyapatite phantom to monitor image brightness and noise and for adjustment of scanner differences in brightness during reading, and to allow comparability of scans among sites. A radiologist identified the courses of the coronary arteries, using specially developed image-processing software programmed to define a calcific focus as four adjacent pixels comprising an area of at least 1.87 mm^2. Agatston scores calculated for each artery (left main, left anterior descending, left circumflex, and right coronary artery) were summed across all arteries to obtain the total calcium score used in all analyses *(24)*. Each participant received two scans. Each scan set with at least one non-zero score and a random sample of those with zero scores were reviewed side by side by an expert investigator (Dr. Robert Detrano, Division of Cardiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA) without knowledge of the Agatston scores to verify the presence of coronary calcium. Scan sets that were both positive or both negative were confirmed. Of 107 scan sets with one nonzero score and one zero score, 46 were judged negative and 61 were judged positive. For scan sets that were judged positive after review, the overall score was calculated as the mean of the scores of the two scans. For all other scan sets, the overall score of the scan set was recorded as zero. The method of standardization between methods (EBCT and multidetector CT) appeared effective because we found no difference in the incidence of detected CAC among sites, adjusted for age, sex, and race. The reading method appeared both reliable and stable given a pilot study of 379 individuals at the year 10 CARDIA examination, with scans that were read identically to those collected at year 15. Fifteen individuals were positive for coronary calcification on both occasions, 13 became positive at year 15, and 4 with low Agatston scores lost positivity at year 15 (of whom, on rereading the year 10 scans, 2 were judged falsely positive). Except for these 4 and the 347 people without coronary calcification, Agatston scores increased in each individual over the 5-year period.

STATISTICAL ANALYSIS

Variables potentially associated with plasma concentrations of F_2-isoprostanes were measured at the year 15 examination unless otherwise indicated. Variables were screened by use of correlation coefficients and by computing mean concentrations of F_2-isoprostanes within categories of each potential correlate. Variables with significant ($P < 0.05$) correlations were evaluated further by linear regression adjusted for race, gender, age, and clinical center. Because 47% of participants with any CAC had a low Agatston score of <20 (83% had an Agatston score <100), primary analyses used CAC presence or absence as the dependent variable to ascertain the proportion of participants who had any CAC for each sex group within quartiles of F_2-isoprostanes (computed across the total population). Logistic regression was then used to quantify odds ratios for having CAC per standard deviation of F_2-isoprostanes. Initial models were adjusted only for race, gender, age, and center. Final multivariate models were adjusted for age, race, sex, race-sex interactions, BMI (linear and quadratic terms plus all possible interactions of these with race and sex), clinical site, systolic blood pressure, use of blood pressure-lowering or cholesterol-lowering medication, current smoker, ex-smoker, HDL-C, LDL-C, ln(triglycerides), antioxidant vitamin use, impaired fasting glucose, diabetes, and ln(CRP+1). The race/gender-specific quadratic functions of BMI were used because the pattern of mean F_2-isoprostanes was well-fitted by quadratic functions of BMI (data not shown). We ran parallel linear regression analyses using the ln(Agatston score + 1) as the dependent variable.

Results

In the comparison of cardiovascular risk factors (Table 1), men had higher plasma concentrations of LDL-C and triglycerides and lower HDL-C and CRP concentrations than women. Slightly more men were current smokers, and men had higher blood pressures. The mean F_2-isoprostane concentrations were significantly greater in women than men (190.0 vs 140.4 pmol/L), with the following percentile cutoffs for women and men, respectively: 5th [81.5 and 73.8 pmol/L (28.9 and 26.2 ng/L)], 25th [117.7 and 103.4 pmol/L (41.7 and 36.7 ng/L)], 50th [161.7 and 128.5 pmol/L (57.3 and 45.6 ng/L)], 75th [228.3 and 168.6 pmol/L (81.0 and 59.1 ng/L)], and 95th [387.2 and 249.2 pmol/L (137.4, 88.4 ng/L)]. The prevalence of any CAC was threefold higher in men than women. Nevertheless, the median Agatston score among those

Table 1. Selected characteristics of gender groups at the year 15 examination in CARDIA.[a]

Characteristic	Men (n = 1302)	Women (n = 1548)	P
Age, years	40.2 (3.6)	40.4 (3.6)	0.19
BMI, kg/m^2	28.0 (4.9)	28.9 (7.2)	0.001
Blood pressure, mmHg			
Systolic	115.1 (13.6)	111.1 (14.8)	<0.0001
Diastolic	76.4 (11.1)	72.7 (11.1)	<0.0001
Blood pressure medication use, %	7.0	7.0	0.53
Current smoking, %	22.0	19.0	0.03
Triglycerides, mmol/L [mg/L]	1.3 (0.8) [1144 (686)]	1.0 (0.5) [867 (481)]	<0.0001
HDL-C, mmol/L [mg/L]	1.2 (0.3) [453 (126)]	1.4 (0.4) [553 (142)]	<0.0001
LDL-C, mmol/L [mg/L]	3.1 (0.9) [1193 (332)]	2.8 (0.8) [1090 (292)]	<0.0001
CRP,[b] mg/L	1.6 (2.43)	2.3 (2.9)	<0.0001
F_2-Isoprostanes, pmol/L [ng/L]	140.4 (55.6) [49.8 (19.7)]	190.0 (108.9) [67.4 (38.6)]	<0.0001
CAC, % positive (n)	15.0 (190)	5.0 (79)	<0.0001

[a] Values are the mean (SD) except for blood pressure medication use, current smoking, and CAC.
[b] CRP cannot be expressed in molar concentrations because of the presence of multiple molecular forms.

with any CAC did not vary significantly by sex (16.9 for women and 22.3 for men).

Mean F_2-isoprostane concentrations did not vary significantly in either men or women with a BMI <25.0 kg/m^2, but they increased steadily from 25 kg/m^2 through >40 kg/m^2. The increase with BMI was significantly greater among women than among men. The F_2-isoprostane concentrations differed between men and women who were within the same BMI category (20 categories were compared across the full BMI range). Women had higher values in all categories.

Partial correlations of F_2-isoprostanes, adjusted for race and sex, were $r = 0.16$ with ln(CRP+1), $r = 0.14$ with ln(triglycerides), $r = 0.12$ with systolic blood pressure, and $r = 0.09$ with current smoking ($P < 0.001$ for each correlation), but there were no significant correlations with LDL-C. Except for the association with smoking, these correlation coefficients were strongly attenuated or negated entirely after adjustment for the race/gender group-specific quadratic function of BMI. One exception was the finding of an association between HDL and F_2-isoprostanes ($r = 0.13$) only after adjustment for BMI.

Within each sex group, individuals with the highest F_2-isoprostane concentrations had the highest observed prevalence of CAC with increases more apparent for men than women (Fig. 1). In men, CAC prevalence increased from quartile 1 to quartiles 2 and 3, followed by a somewhat larger increase between quartiles 3 and 4. In women, the F_2-isoprostane concentrations remained relatively flat across quartiles 1–3, followed with an increase in quartile 4. In a logistic regression model adjusted for race, gender, age, and center (Table 2A), the odds ratio for the presence of CAC was 1.24 per 92.2 pmol/L (32.7 ng/L) of F_2-isoprostanes (1 SD).

The results of sex-specific models were consistent with the overall model, with the odds of having CAC of 1.25 per 92.2 pmol/L (32.7 ng/L) of F_2-isoprostanes [95% confidence interval (CI), 1.08–1.44] for men and 1.19 per 92.2 pmol/L (32.7 ng/L; CI, 0.99–1.44) for women. In the fully adjusted model, the results were 1.19 per 92.2 pmol/L (32.7 ng/L; CI, 1.01–1.40) for men (190 CAC cases) and 1.13 per 92.2 pmol/L (32.7 ng/L; CI, 0.89–1.44) for women (79 CAC cases).

In linear regression analyses of the overall model (Table 2B), the Agatston score increased by 6.9% per 92.2 pmol/L (32.7 ng/L) of F_2-isoprostanes, whether in the minimally adjusted ($P = 0.0007$) or final ($P = 0.0077$) multivariate model. The same analysis did not find a significant relationship among only those individuals with CAC.

Discussion

Plasma F_2-isoprostane concentrations are an independent predictor for the presence of CAC and are differentiated between those with no CAC and those with the generally low Agatston scores found in the CARDIA population.

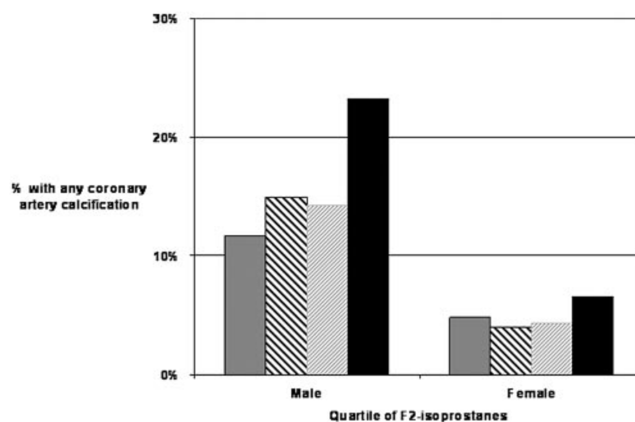

Fig. 1. Percentage of participants with CAC by quartile of F_2-isoprostanes.

A total of 168 men and 560 women were in the highest quartile, with 315–412 participants in the remaining categories. F_2-Isoprostane quartiles: ■, <108.6 pmol/L (38.5 ng/L); ▧, 108.9–143.5 pmol/L (38.6–50.9 ng/L); ▨, 143.8–196 pmol/L (51–69.5 ng/L); ■, >196.3 pmol/L (69.6 ng/L).

Table 2. Regression analysis of CAC per standard deviation of F_2-isoprostanes (32.7 ng/L).

A. Logistic regression (dependent variable, presence of CAC)

	Odds ratio	95% CI
Adjusted for race, sex, age, and center	1.24	1.09–1.41
Fully adjusted[a]	1.18	1.02–1.38

B. Linear regression [dependent variable, ln(Agatston score + 1)]

	Change in Agatston score per SD of F_2-isoprostanes, %	95% CI, %
All participants (n = 2850)		
Adjusted for race, sex, age, and center	6.9	2.8–11.1
Fully adjusted[a]	6.0	1.6–10.7
Participants with any CAC (n = 269)		
Adjusted for race, sex, age, and center	15.7	−4.9 to 39.7
Fully adjusted[a]	24.2	−2.1 to 57.5

[a] Fully adjusted model includes age, race, sex, race × sex, BMI (linear and quadratic plus all possible interactions of these with race and sex), clinical site, systolic blood pressure, use of blood pressure-lowering or cholesterol-lowering medication, antioxidant supplement usage, diabetes, impaired fasting glucose, current smoker, ex-smoker, HDL-C, LDL-C, ln(triglycerides), and ln(CRP + 1).

The association of F_2-isoprostanes with CAC was independent of the primary lipoproteins involved in atherosclerosis and the CRP concentration, a marker of ongoing inflammatory processes, as well as many other cardiovascular disease risk factors *(9)*. Together, these observations indicate that plasma F_2-isoprostanes may be an independent indicator for the risk of CAC in generally healthy populations.

In this study, individuals were 33–45 years of age with little clinical cardiovascular disease. Only 10% of the population had any CAC, and among these individuals the amounts of CAC were very low compared with populations having clinical coronary heart disease *(25)*, implying a population in the early stages of subclinical cardiovascular disease. For comparison, a typical score is >300 for individuals with diagnosed cardiovascular disease. Nearly 50% of individuals in CARDIA who had CAC had an Agatston score <20.

The association of plasma F_2-isoprostane concentrations with CAC in these generally healthy young participants suggests that lipid peroxidation may have an integral and initiating role in the early development of plaque. F_2-Isoprostanes are an indicator of systemic oxidative damage and have been found in almost all tissues *(23)*. The low concentrations of F_2-isoprostanes usually found in tissues increase rapidly in experimental animals after exposure to carbon tetrachloride *(26)* and Diquat *(27)*, known oxidative agents. In a similar manner, the circulating concentrations of F_2-isoprostanes in humans increase or decrease in response to oxidative stressors and antioxidants *(9, 13)*. Both genetic and environmental factors can influence F_2-isoprostane concentrations. Plasma concentrations of F_2-isoprostanes are increased in smokers, individuals with low antioxidant or antioxidant enzyme concentrations, and individuals with certain genetic disorders *(12, 28–30)*. Thus, they are sensitive and reliable indicators of systemic oxidative damage *(13, 26, 27)*. Moreover, changes in blood concentrations of F_2-isoprostanes occur over relatively long periods of time in humans, requiring ~2 weeks for a 35% decrease after smoking cessation *(13)*.

F_2-Isoprostanes have been found in the oxidation products of arachidonic acid, oxidized LDL particles *(30)*, and atherosclerotic plaques *(9)*. Although the findings reported here are consistent with an integral role for F_2-isoprostanes in the pathogenesis of atherosclerosis, the amounts of F_2-isoprostanes released during the early development of atherosclerotic plaque appear unlikely to account for the circulating F_2-isoprostanes reported in this study. For example, even those individuals without detectable CAC had measurable concentrations of plasma F_2-isoprostanes, and mature atherosclerotic plaques are estimated to contain F_2-isoprostanes at a concentration of only 76.4 (60.4) pmol/g [27.1 (21.4) ng/g] of plaque (wet weight) *(31)*. Even the release of all F_2-isoprostanes contained in plaque would not account for all of the plasma F_2-isoprostanes [3000 mL (typical plasma volume) × 166.4 pmol/L (59 ng/L; mean concentration in CARDIA) = 499.1 pmol (177 ng), which would correspond to 6.5 g of plaque, more than is likely to exist in these participants]. In addition, the increased F_2-isoprostane concentrations are not a consequence of poor excretion as a result of impaired renal function: those individuals were excluded from the analysis. Instead, the plasma concentrations of F_2-isoprostanes appear to identify individuals with increased overall oxidative stress, which in turn is associated with an increased risk of oxidation of extracellular and circulating lipids, among which are LDL particles.

Coronary calcification is not only correlated with atherosclerosis; it is a subset of atherosclerosis and is found in a particularly problematic anatomic location. The finding of an association between plasma F_2-isoprostane concentrations and CAC has clear etiologic importance in identifying oxidative damage as being involved in the early stages of atherosclerosis. A prospective study of 17 256 individuals found a progressive increase in the risk of combined fatal and nonfatal coronary heart disease from the lowest to highest quartile of CAC *(31)*. The Agatston score correlated well with age in autopsy studies of people with coronary heart disease *(32)*. Although not all atherosclerotic segments have detectable calcification, the area of CAC has been shown to correlate well with the histopathologic coronary plaque area *(33)*. Several outcome studies indicated that coronary calcification may be an independent predictor of angiographic coronary artery disease *(34)* and of future coronary events *(35)*. Together, these studies suggest that CAC measurements are an intermediary marker for future cardiovascular disease events.

In agreement with a smaller study *(36)*, women had

higher F_2-isoprostanes than did men, but women had a lower prevalence of CAC than men. This situation seems paradoxical, but it occurs for other major risk factors of cardiovascular disease, including blood cholesterol concentrations and fibrinogen, an acute-phase reactant and clotting factor. Women have higher concentrations of plasma fibrinogen than men but a lower risk of cardiovascular disease *(37, 38)*. The risk of coronary heart disease associated with fibrinogen diminishes with age in women but not in men. These results indicate a gender difference in responsiveness toward the risk factor that may be modulated by interactions with other gender-specific factors. In a similar manner, women have higher HDL concentrations than men, and changes in HDL may have a greater impact on the risk of cardiovascular disease in women than men *(39)*. The National Cholesterol Education Program recognizes a possible differential impact of changes in HDL concentrations on men and women *(40)* and proposes more aggressive guidelines for women than men. The current isoprostane results support the possibility of a differential response in F_2-isoprostane formation between men and women and that women may have more protection against the effects of oxidation or may metabolize lipids differently from men. Although the observed relationship between F_2-isoprostanes and CAC among women alone might be attributable to a chance observation; this relationship was still significantly positive, consistent with that observed among men (odds ratios, 1.13 for women and 1.19 for men in the fully adjusted model). Moreover, the finding of an increased incidence of CAC, primarily in the fourth quartile of F_2-isoprostane concentrations for women, is consistent with the very low amounts of atherosclerotic plaque found in autopsies of young women compared with young men. Further research should consider whether antioxidant protection systems, and their interaction with lipid metabolism, differ between men and women.

In conclusion, our findings emphasize the strong relationships between concentrations of circulating F_2-isoprostanes and the early development of coronary artery disease, as measured by CAC. Furthermore, these observations suggest increased production of oxidized products relating directly to the risk of coronary disease, thereby supporting the proposition that a general state of oxidative stress plays a role in the etiology of cardiovascular disease.

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