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Oxidative stress and insulin resistance: The CARDIA study

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Background: Although cumulative evidence suggests that increased oxidative stress may lead to insulin resistance *in vivo* or *in vitro*, community-based studies are scarce.
Objective: This study examined the longitudinal relationships of oxidative stress biomarkers with development of insulin resistance and whether these relationships were independent of obesity in non-diabetic young adults.

Research design and methods: Biomarkers of oxidative stress (F_2-isoprostanes (F_2Isop) and oxidized LDL (oxLDL)), insulin resistance (the homeostasis model assessment of insulin resistance (HOMA-IR), and various fatness measures (Body Mass Index (BMI), waist circumference (WC), and estimated percent fat) were obtained in a population-based observational study (Coronary Artery Risk Development in Young Adults (CARDIA)) and its ancillary study (Young Adult Longitudinal Trends in Antioxidants (YALTA)) during 2000-2006.

Results: There were substantial increases in estimated mean HOMA-IR over time. OxLDL and F_2Isop showed little association with each other. Mean evolving HOMA-IR increased with increasing levels of oxidative stress markers ($P<.001$ for oxLDL and $p=0.06$ for F_2Isop), measured in 2000-01. After additional adjustment for adiposity, a positive association between oxLDL and HOMA-IR was strongly evident, whereas association between F_2Isop and HOMA-

Conclusion: We observed positive associations between each of two oxidative stress markers and insulin resistance. The association with oxidized LDL was independent of obesity, but that with F_2Isop was not.

Clinical type 2 diabetes is considered to be preceded by a long period of insulin resistance, during which blood glucose is maintained at near normal levels by compensatory hyperinsulinemia (1). Convincing evidence has established that the level of insulin resistance is a prediabetic state that can predict incident type-2 diabetes relatively far into the future (2).

Increased oxidative stress appears to be a deleterious factor leading to insulin resistance, β-cell dysfunction, impaired glucose tolerance, and ultimately, to type 2 diabetes (3,4). Obesity may play a role in the relationship between systemic oxidative stress and these conditions (5). Chronic oxidative stress is particularly dangerous for β-cells because pancreatic islets are among those tissues that have the lowest levels of antioxidant enzyme expression, and β-cells have high oxidative energy requirements (4). In addition, there is considerable evidence that increased free radicals impair glucose-stimulated insulin secretion, decrease the gene expression of key β-cell genes, and induce cell death (4,6). If β-cell functioning is impaired, it results in an underproduction of insulin, fasting hyperglycemia, and eventually, the development of type-2 diabetes (7).

However, most previous studies investigating this association have been in vitro or in small in vivo studies (8,9), and data presenting an association between the degree of oxidative stress and the risk of developing insulin resistance among non-diabetic people in the community are scarce (9). We explored the associations between oxidative stress and insulin resistance to see whether elevated levels of oxidative stress markers increase the risk of future insulin resistance, whether different biomarkers of oxidative stress show consistent results, and whether these associations can be explained by obesity in a longitudinal design in a population-based cohort. We elected to study F_2-isoprostanes (F_2Isop) (10) and oxidized LDL (oxLDL) (11-13), which mark complementary areas of systemic oxidative stress. In order to strengthen our hypothesized direction from oxidative stress to insulin resistance, we tested the reciprocal relationship to see if elevated levels of HOMA-IR were associated with increase in the level of one oxidative stress marker longitudinally.

METHODS

Subjects and measurements: Data from the Coronary Artery Risk Development in Young Adults (CARDIA) and its ancillary study, Young Adult Longitudinal Trends in Antioxidants (YALTA), were used to examine the association between biomarkers of oxidative stress and insulin resistance. Briefly, CARDIA is a longitudinal study aiming to investigate lifestyle and other factors that influence the evolution of cardiovascular disease (CVD) in young adults. This study began in 1985 with a cohort of 5,115 healthy black and white men and women, aged 18-30 years, who were free-living individuals residing in 4 US cities (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). The YALTA ancillary study analyses blood and urine biomarkers of oxidative stress, endothelial dysfunction, and related concepts, and interprets those data in conjunction with the other data that have been collected by CARDIA. Follow-up examinations were conducted during 1987-1988 (Year 2), 1990-1991 (Year 5), 1992-1993 (Year 7), 1995-1996 (Year 10), 2000-2001 (Year 15), and 2005-2006 (Year 20). The percentages of the surviving cohort who returned for these examinations were 90%, 86%, 81%, 79%, 74%, and 72%, respectively. In this analysis, we included all participants with F_2Isop or oxLDL measurements at Year

15 and fasting insulin/fasting glucose measurements at Year 20. Among them, we excluded those who had been diagnosed with diabetes, defined as a fasting glucose of ≥126 mg/dl (7 mmol/L) or who were receiving antidiabetic medication between Years 0 and 20 in our analysis. For additional sub-group analysis, examining the age pattern of the homeostasis model assessment of insulin resistance (HOMA-IR) with the level of year 15 oxLDL, we included those who had both year 15 oxLDL and HOMA-IR at years 0, 7, 10, 15, and 20.

Self-reported demographic (age, sex, race, educational level) and behavioral (smoking, alcohol consumption, physical activity) information was obtained across CARDIA examination visits. Educational status was quantified as the reported number of years of schooling completed. Self reported alcohol beverages were quantified as average milliliters of alcohol consumed per day, and smoking status was classified as never, former, or current smoker. An interviewer-administered questionnaire queried participation spent in leisure, occupational, and household physical activities over the course of a year, weighting frequency and intensity in order to obtain a total activity score.

Anthropometry: The participants wore light clothes without shoes during anthropometry. Body weight (WGT) was recorded to the nearest 0.2 pound. Body height (HGT) was recorded to the nearest 0.5 centimeters, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, using the average of the two measurements. Waist circumference (WC, cm) was measured with each participant's weight distributed equally on both feet. The measurement was made midway between the iliac crest and the lowest lateral portion of the ribcage and anteriorly midway between the xyphoid process of the sternum and the umbilicus, keeping the tape (vinyl anthropometric centimeter tape) horizontal.

Since the adiposity measurements, total fat mass and percent fat, were obtained only from a sub-sample (n=1072) of the study participants, race- and sex- specific predictive equations for percent body fatness (%FAT) were computed using the Year-10 dual energy x-ray absorptiometry (DXA) examination (14); these estimates enabled study of total body fatness of each participant. Year 10 DXA was obtained only from a sub-sample (n=1072), in Birmingham and Oakland, of the study participants using the QDR 2000 (Hologic, Bedford, MA) with software version 11.1. These race-sex-specific predictive equations included age, BMI, WC, and squared terms for waist circumference and BMI (WC^2 and BMI^2). For black men, the equation was: $\%FAT = -55.5371 - 0.0578*AGE + 0.1585*BMI + 1.1741*WC - 0.00365*WC^2$; for black women: $\%FAT = -52.0734 + 0.0901*AGE + 1.9772*BMI + 0.9664*WC - 0.015*BMI^2 - 0.0051*WC^2$; for white men: $\%FAT = -82.6174 + 0.1015*AGE + 0.5448*BMI + 1.5883*WC - 0.0065*WC^2$; and for white women: $\%FAT = -75.2741 + 0.1698*AGE + 1.2678*BMI + 1.7154*WC - 0.00972*WC^2$. These equations had higher correlation coefficients and smaller mean square errors and predictive errors compared to the widely used general predictive equations for body composition, such as the Durnin and Womersley equations (15). The correlations of observed Year 10 percent fat with predicted values were R^2: 0.66 for black men, 0.76 for black women, 0.60 for white men, 0.74 for white women, and 0.82 for sex and race combined, compared to R^2 about 0.75 for the Durnin and Womersley equations that relied solely on skinfolds. These formulae closely predicted DXA measured fat and lean mass at year 20.

Blood components: Before drawing blood, all of the participants were asked to fast for 12 hours and to abstain from smoking for two hours before their examinations. After

venous blood samples were drawn, plasma was separated by centrifugation, transferred into airtight vials, stored at -70 ºC and shipped on dry ice.

Fasting glucose and insulin were measured at years 0, 7, 10, 15, and 20. Glucose was measured in stored blood samples using the hexokinase ultraviolet method on a Cobas Mira Plus chemistry analyzer. The insulin measurements were performed by using a radioimmunoassay (RIA) with an overnight, equilibrium-incubation format. Based on re-assays of glucose in December 2007 in about 200 samples stored since year 7 and 100 samples stored since year 15, glucose and insulin were recalibrated to harmonize them with the previous measurements. Recalibrated glucose values were 6.98 + 0.94 * year 7 glucose concentration, 7.15 + 0.96 * year 10 glucose concentration, 6.99 + 1.01 * year 15 glucose concentration, 4.06 + 0.97 * year 20 glucose concentration. Recalibrated insulin was -0.36 + 0.93* year 20 insulin concentration.

The biomarkers of oxidative stress were obtained at Year 15 and/or Year 20. F_2Isop was measured at Years 15 and 20 with gas chromatography-mass spectrometry. OxLDL concentrations were measured by the Mercodia oxidized LDL ELISA (Mercodia AB, Uppsala, Sweden) in Year 15 samples that had been stored at -70^{o}C for several years. The antibody (mAb-4E6) is directed against a conformational epitope in the apolipoprotein B-100 moiety of LDL, as a consequence of substitution of ≥ 60 lysine residues of apolipoprotein B-100 with aldehydes, and these substituting aldehydes can be produced by peroxidation of lipids of LDL, leading to the generation of ox-LDL. This method was compared with another assay, developed by Holvoet et al. (11), and showed the similar analytical performance and clinical applicability (11).

Plasma levels of ascorbic acid were measured using high performance liquid chromatography (HPLC) at Year 15. Levels of carotenoids (including lycopene, α-carotene, ß-carotene, ß-cryptoxanthin, and zeaxanthin plus lutein) and tocopherols (α-tocopherol and γ-tocopherol) were obtained by HPLC at Year 15. A common protocol and quality-control procedures were used for all examinations.

Statistical analysis: The BMI and WC were set to missing at each examination at which a woman was pregnant. We investigated patterns of repeated measurements for individuals with large within person standard deviations from year 0 to year 20 by visual inspection of the raw data to detect if there were any substantial departures (e.g., outliers) from patterns. Six outliers of WC at Year 15, one outlier of BMI at Year 20, one outlier of F_2Isop at Year 15, and one outlier of HOMA-IR at Year 7 were replaced with missing values. Insulin resistance was assessed by the homeostasis model assessment of insulin resistance (HOMA-IR), which was calculated as fasting plasma insulin (mU/L) * fasting plasma glucose (mmol/L) / 22.5 (16). HOMA-IR, F_2Isop, oxLDL and circulating antioxidant (ascorbic acid, lycopene, α-carotene, ß-carotene, ß-cryptoxanthin, zeaxanthin plus lutein, α-tocopherol and γ-tocopherol) estimates were logarithmically transformed, since their distributions were skewed to the right. The subjects were classified into quartiles, based on their levels of oxidative stress markers.

Partial correlation coefficients between body fat measurements/antioxidants and oxidative stress markers were calculated by sex, adjusting for age and race.

Multivariable generalized linear models (GLM) were used to test associations of oxidative stress markers with evolving HOMA-IR, using as dependent variable HOMA-IR at year 20 and adjusting for demographic and lifestyle factors (e.g., age, sex, race, study center, smoking status,

physical activity, alcohol consumption, education), and the previous measure of HOMA-IR at Year 15. In addition, the models were adjusted for adiposity measurements (e.g., BMI, WC, and equation-driven estimates of percent fat) to examine if the association between oxidative stress and insulin resistance was independent of obesity. All analyses were conducted using SAS version 9.1.

RESULTS

The mean age of the participants during 2000-2001 (Year 15) was 40 years and 45% were men among 2774 non-diabetic study participants. Participants who had higher levels of F_2Isop were more likely to be female ($p<0.001$, Table 1). In contrast, women had lower levels of oxLDL ($p<0.001$). Education showed an inverse association with both markers ($p<0.001$). An increased level of F_2Isop was positively associated with smoking ($p<0.001$), whereas oxLDL was not associated with this factor. Physical activity was inversely associated with both markers, though the gradient was stronger over F_2Isop than over oxLDL.

Body fat measurements showed strong positive associations with both markers ($p<0.01$), as shown in Table 1. F_2Isop showed much stronger correlations with adiposity markers in women than in men (Table 2). Correlations between F_2Isop and body fat measurements, including BMI, WC and estimated body percent fat, were 0.40-0.43 in women, whereas these correlations were only 0.15-0.16 in men, after adjustment for race and age. OxLDL did not show a sex difference in the correlations with body fat measurements. F_2Isop was negatively correlated with sum of 5 serum carotenoids, ascorbic acid and alpha tocopherol, but positively correlated with serum gamma tocopherol. In contrast, the correlations between oxLDL and antioxidants were minimal. Interestingly, the two oxidative stress markers, Isop and oxLDL, were minimally correlated in our sample ($r=0.09$, $P=0.0007$ in women and $r=-0.003$, $P=0.9$ in men).

Table 3 includes adjusted estimates for year 20 HOMA-IR, according to the quartiles of year 15 F_2Isop and oxLDL. We observed a significant increase in the estimates of evolving HOMA-IR with increasing levels of oxLDL ($P<.001$) and a marginally significant association with F_2Isop ($P=0.06$). After additional adjustment for the adiposity measurements, oxLDL was still significantly associated with year 20 HOMA-IR, whereas the association between F_2Isop and HOMA-IR became substantially attenuated. Further adjustments for HDL-cholesterol, LDL-cholesterol, and triglycerides did not affect the magnitude and significance of the association between oxLDL and HOMA-IR. Adiposity measurements remained significant predicting HOMA-IR in the models adjusted for either marker of oxidative stress ($p<0.0001$).

In our analysis, we did not adjust for medication use (e.g., lipid or blood pressure lowered medications) because such use could mediate or be directly correlated with mediators in the causal pathway for associations of oxidative stress on insulin resistance. Omission of those taking these medications did not materially change our study results (data not shown).

There were no significant interactions between oxidative stress markers and other covariates, including sex, race, smoking, adiposity measurements, and physical activity. For example, although the association between F_2Isop and HOMA-IR was positive in whites and negative in blacks, race specific coefficients were small and p-values for interactions were not significant.

Increase in F_2Isop was not predicted by HOMA-IR in longitudinal analysis, controlling for body fat measurements (BMI, WC or estimated body percent fat), age, sex,

race, study center, smoking status, physical activity, alcohol consumption, education, and F_2Isop, all of which were measured in the same year as the independent variable. (BMI adjusted regression coefficient = -0.004, p=0.9; WC adjusted regression coefficient = -0.009, p=0.7; body fat percent adjusted regression coefficient = -0.008, p=0.7).

DISCUSSION

We observed positive associations between oxidative stress markers and insulin resistance in a non-diabetic adult population. OxLDL was positively associated with insulin resistance, even after accounting for various adiposity measurements, a finding akin to a recent report in these data that oxLDL predicted incident metabolic syndrome (12). Although the association between F_2Isop and insulin resistance was also positive, it was explained by additional adjustment for adiposity measurements. The levels of Year 20 HOMA-IR predicted by each oxidative stress marker were not significantly different by sex. Recently Meigs et al. showed in a cross-sectional design that the association between HOMA-IR and oxidative stress, measured by urinary isoprostanes (8-epi-PGF 2 α/creatinine), was significant, independent of BMI, but association between insulin resistance prevalence and oxidative stress was attenuated by additional adjustment for BMI (p=0.06) in individuals without type 2 diabetes in the Framingham Offspring Cohort study (9). Our study substantially extends what is known about the impact of oxidative stress on insulin resistance in people without diabetes. In our longitudinal analysis, the results show that plasma F_2Isop was marginally related with elevated levels of HOMA-IR, but a substantial part of this association was explained by anthropometric measurements (BMI and WC). Furthermore, we examined this association by using an alternate marker, oxLDL, that showed a stronger association with insulin resistance, independent of body fatness, compared to F_2Isop.

Oxidative stress results from an imbalanced condition in which the generation of free radicals is greater than the capacity of the antioxidant defense system to detoxify them (17). When oxidative stress is chronic, it is thought to result in damage to DNA, lipids, proteins and other molecules, which may contribute to the development and progression of chronic disease, including cardiovascular disease and cancer (13,18). Recently various indirect markers of oxidative stress have been used in epidemiological studies to measure oxidation damage due to feasibility and cost issues, instead of direct measures of free radicals and ROS, which include electron resonance or spin trapping, capturing free radical reactions in real time (19). Among them, oxLDL and F_2Isop have been known as robust reflectors of oxidative stress in humans and have been widely used in biology, medicine and epidemiological research (20,21). Each marker may capture different stages of the oxidative process or reflect different pathophysiological pathways (22). The apparent compartmentalization of oxidative stress is illustrated in the differing associations of F_2Isop, oxLDL, and different carotenoids with each other and with HOMA-IR. There was little correlation between the two measures of oxidative stress, though F_2Isop was well correlated with the sum of serum carotenoids and other serum antioxidant vitamins (e.g., ascorbic acid, and alpha tocopherol), lower values of which in part indicate reduced carotenoid intake and in part oxidative stress. In contrast, correlations between oxLDL and these antioxidant nutrients were substantially weaker. In longitudinal analysis using CARDIA/YALTA, Hozawa et al. (23) reported that serum carotenoid concentrations were inversely associated with incident diabetes and HOMA-IR in nonsmokers, concluding that oxidative stress may be

involved in the development of type 2 diabetes in nonsmokers. It has been reported that serum carotenoids were inversely associated with F_2Isop in the same data (24).

Our study shows that a substantial portion of the association between F_2Isop and HOMA-IR was explained by body fat measurements, and F_2Isop was strongly correlated with adiposity markers, especially in women. In a previous report using CARDIA/YALTA, Gross et al. (10) reported that the level of plasma F_2Isop was higher in women, especially for those with a BMI greater than 25 kg/m^2, than men. Since F_2Isop is a product of lipid peroxidation, it is closely related to adipose tissue mass, especially for women. In contrast, correlations between oxLDL and body fat measurements did not differ by sex. Furthermore, oxLDL was a strong precursor of the risk of insulin resistance, independent of obesity. Therefore, we suggest that each oxidative stress marker may capture a different oxidative stress pathway and their interaction with adiposity differ between men and women. Nevertheless, in our study the estimated levels of evolving HOMA-IR with increasing levels of the oxidative stress markers did not differ significantly by sex.

Our study has several strengths. Studies examining the longitudinal associations between oxidative stress and insulin resistance are rare, especially with plasma oxidative stress markers in healthy young people in large community-based studies, while most previous epidemiological studies have been used urinary markers. Also, CARDIA has maintained a high participation rate and high-quality data collection through rigorous quality control procedures throughout the study. Furthermore, we could test prediction in the reverse direction and added to the specificity of our finding by showing that HOMA-IR did not predict future oxidative stress, measured by F_2Isop, although we only measured oxLDL once and so were not able to measure the reverse direction with it.

Among potential limitations, first, oxidative stress is a highly complex phenomenon and our measures may not be sufficiently detailed to fully understand the role of oxidative stress and adipose tissue in development of insulin resistance. Second, the study was conducted only among black and white individuals, with no representation of Hispanic, Asian, or other individuals. As a result, the conclusions from this study may not be applicable to all populations. Finally, there could be residual confounding due to this study's observational nature.

In summary, our results show that increased oxidative stress is associated with evolution of insulin resistance. The independent association of oxLDL with insulin resistance supports the hypothesis by which oxidative stress leads to insulin resistance, independent of obesity. F_2Isop is closely correlated with adiposity, especially for women, and it is possible that F_2Isop is intermediate in a pathway that links insulin resistance with total body fat. Further research is needed to elucidate the underlying etiological relationships between adiposity and oxidative stress in the genesis and progression of insulin resistance by various biomarkers of oxidative stress and body fat measurements in diverse populations.

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***Table 1**--Means (or proportions) of demographic, lifestyle, and type 2 diabetes related factors by quartile of oxidative stress Markers*[3]

2000-01 variable (except where indicated)	Quartiles of year 15 F_2-isoprostanes					Quartiles of year 15 oxidized LDL				
	1st quartile	2nd quartile	3rd quartile	4th quartile	P trend	1st quartile	2nd quartile	3rd quartile	4th quartile	P trend
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)		Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	
N	693	693	695	693		652	650	652	652	
Demographics										
Age (years)	40.3(3.5)	40.2(3.5)	40.2(3.6)	40.0(3.7)	0.2	40.1(3.6)	40.2(3.6)	40.2(3.5)	40.2(3.7)	0.9
White, %	58.3	55.8	57.8	54.6	0.4	62.0	56.6	55.8	53.2	0.01
Men, %	57.1	56.0	46.3	22.4	<.0001	35.7	39.9	50.8	55.5	<.0001
Education (grade of school completed)	15.3(2.6)	15.1(2.5)	15.0(2.5)	14.7(2.4)	<.0001	15.4(2.6)	15.0(2.5)	15.0(2.5)	14.8(2.4)	<.0001
Lifestyle										
Alcohol intake (g/day)	9.2(24.8)	10.3(18.2)	12.3(24.0)	13.1(33.1)	0.05	11.5(21.5)	9.9(20.0)	11.9(31.3)	12.0(28.5)	0.6
Physical activity (exercise units)	399(295)	394(295)	357(286)	286(253)	<.0001	382(289)	354.4(283)	364(292)	339(270)	0.01
Current smoker, %	14.5	19.5	21.9	25.4	<.0001	19.2	20.4	19.2	22.0	0.5
Body fat components										
Waist circumference (cm)	85.4(12.3)	86.7(11.8)	87.7(13.1)	91.6(14.3)	<.0001	81.9(12.1)	86.3(13.1)	89.4(12.3)	93.7(12.0)	<.0001
BMI (kg/m2)	26.2(4.7)	27.0(4.7)	27.9(5.9)	31.0(7.2)	<.0001	25.9(5.4)	27.9(6.4)	28.5(5.5)	29.9(5.8)	<.0001
Estimated body percent fat (%)	29.6(8.6)	30.7(9.2)	33.3(10.1)	40.3(10.5)	<.0001	31.9(9.9)	33.9(10.8)	33.5(10.6)	34.5(10.4)	<.0001
Oxidative stress markers										
ln (F2-isoprostanes, ng/L)	3.47(0.2)	3.81(0.1)	4.09(0.1)	4.58(0.3)	<.0001	3.98(0.5)	4.00(0.4)	3.98(0.5)	4.0(0.5)	0.7

ln (oxidized LDL,U/L)	4.32(0.3)	4.36(0.3)	4.32(0.4)	4.33(0.3)	0.7	3.89(0.2)	4.25(0.1)	4.47(0.1)	4.73(0.1)	<.0001
ln (sum of 5 carotenoids, µg/dl) [1,2]	4.74(0.4)	4.64(0.4)	4.55(0.4)	4.41(0.4)	<.0001	4.63(0.4)	4.59(0.4)	4.58(0.4)	4.54(0.4)	0.0002
ln (ascorbic acid, µg/dl)	2.29(0.4)	2.21(0.4)	2.15(0.5)	2.10(0.5)	<.0001	2.22(0.5)	2.22(0.4)	2.16(0.5)	2.16(0.4)	0.02
ln (alpha tocopherol, µg/dl) [2]	0.84(0.1)	0.80 (0.1)	0.76(0.1)	0.73(0.1)	<.0001	0.79(0.2)	0.78(0.2)	0.77(0.2)	0.78(0.2)	0.08
ln (gamma tocopherol, µg/dl) [2]	0.19(0.1)	0.21(0.1)	0.22(0.1)	0.24(0.1)	<.0001	0.21(0.1)	0.21(0.1)	0.21(0.1)	0.22(0.1)	0.008
HOMA-IR (µU/L*mmol/L)										
ln (HOMA-IR) at year 0	1.09(0.3)	1.11(0.3)	1.11(0.3)	1.14(0.3)	0.0008	1.06(0.3)	1.12(0.3)	1.12(0.3)	1.15(0.3)	<.0001
ln (HOMA-IR) at year 7	1.26(0.4)	1.26(0.3)	1.27(0.3)	1.33 (0.4)	<.0001	1.17(0.3)	1.28(0.4)	1.28(0.3)	1.38(0.4)	<.0001
ln (HOMA-IR) at year 10	1.23(0.3)	1.26(0.3)	1.27(0.4)	1.33(0.4)	<.0001	1.17(0.3)	1.26(0.3)	1.29(0.4)	1.36(0.4)	<.0001
ln (HOMA-IR) at year 15	1.24(0.4)	1.27(0.4)	1.31(0.4)	1.40(0.4)	<.0001	1.14(0.3)	1.28(0.4)	1.34(0.4)	1.46(0.4)	<.0001
ln (HOMA-IR) at year 20	1.31(0.4)	1.35(0.4)	1.38(0.4)	1.43(0.4)	<.0001	1.22(0.4)	1.34(0.4)	1.37(0.4)	1.52(0.4)	<.0001

1. Sum of 5 carotenoids: alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein/zeaxanthin, and lycopene.
2. Adjusted for Year 15 HDL-cholesterol, LDL-cholesterol, and triglycerides (25).
3. All variables were measured at Year 15, except as indicated.

***Table 2**--Sex-specific partial correlation coefficients of observed body fat components, estimated body percent fat and antioxidant markers at year 15 with F2-Isoprostanes and oxidized LDL at Year 15.*

Adiposity and oxidative stress markers	F2-isoprostanes				Oxidized LDL			
	N	Women	N	Men	N	Women	N	Men
Body Mass Index (kg/m^2) [2]	1509	0.43	1260	0.15	1418	0.25	1184	0.23
Waist Circumference (cm) [2]	1506	0.40	1259	0.16	1416	0.31	1183	0.26
Estimated body percent fat (%) [1]	1506	0.41	1258	0.16	1416	0.28	1182	0.26
Sum of 5 carotenoids (□/dl) [2,3,4]	1438	-0.40	1196	-0.27	1382	-0.02 *	1139	-0.06 *
Ascorbic acids (□/dl) [2]	1255	-0.22	1067	-0.24	1206	-0.05 *	1017	0.004 *
Alpha tocopherol (□/dl) [2,3]	1438	-0.29	1196	-0.30	1382	0.02 *	1139	-0.009 *
Gamma tocopherol (□/dl) [2,3]	1438	0.27	1196	0.23	1382	0.03 *	1139	-0.03 *
F2-isoprostane (□/dl) [2]	1513	1	1261	1	1419	0.09	1185	-0.003 *

** p>0.05*

1. Based on race-sex specific prediction from BMI, waist circumference, and age; see Methods

2. All models were adjusted for age, and race

3. Additional adjustment for Year 15 HDL-cholesterol, LDL-cholesterol, and triglycerides (25).

4. Sum of 5 carotenoids: alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein/zeaxanthin, and lycopene.

***Table 3**--Relationship between year 15 oxidative stress markers and year 20 HOMA-IR*

	F2-isoprostanes			Oxidized LDL		
	Beta coefficient	SE	p value	Beta coefficient	SE	p value
Model 1 [1]	0.0244	0.0158	0.1	0.0727	0.0208	0.0005
Model 2 [2]	0.0294	0.0159	0.06	0.0753	0.0208	0.0003
Model 3						
Body Mass Index (kg/m^2) [3]	-0.0192	0.0165	0.2	0.0598	0.0206	0.004
Waist Circumference (cm) [3]	-0.0237	0.0161	0.1	0.0432	0.0206	0.04
Estimated body percent fat (%) [3]	-0.0217	0.0163	0.2	0.0501	0.0206	0.02

Beta coefficient is a change in HOMA-IR (ln(mU/l*mmol/l)) per one unit increment of independent variables (ln(F_2Isop, ng/L) or ln(oxLDL, U/L))

1. Model 1: Adjusting for age, sex, race, study center and Year 15 HOMA-IR.
2. Model 2: Model 1 plus year 15 smoking status, physical activity, alcohol consumption, and education
3. Model 3: Model 2 plus year 15 body fat measurements. (BMI, WC or estimated body percent fat; adding each variable separately)