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Association of blood pressure with fluorescent oxidative products in a study of Whites, African Americans and West African immigrants

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Epidemiology

_____

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By

Baoqing Gong

Bachelor of Medicine
Peking University
2011

Faculty Thesis Advisor: Michael Goodman, MD, MPH

An abstract of
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Abstract

Association of blood pressure with fluorescent oxidative products in a study of Whites, African Americans and West African immigrants
By Baoqing Gong

Background: Oxidative stress defined as an imbalance between pro-oxidant and anti-oxidant factors, leads to cellular damage and may cause disease. Hypertension is thought to be both caused by and result in oxidative stress. Lately, fluorescent oxidation products (FOP) were proposed as a new biomarker of oxidative stress. The purpose of this study was to examine the association between FOP and hypertension in men and women residing in the state of Georgia and representing three racial and ethnic groups: US-born Caucasians, African-Americans and West African immigrants.
Methods: The analyses used simple and multivariable linear regression models to assess the association of FOP to systolic and diastolic blood pressure (SBP and DBP, respectively) and hypertension defined as SBP ≥ 140 mm Hg, or DBP ≥ 90 mm Hg or self reported use of blood pressure lowering medications.
Results: In crude models, the blood pressure measures and hypertension were not associated with FOP concentrations. In the multivariable regression analysis, there was a positively and statistically significant association between FOP and SBP ($\beta = 0.0042$, $p=0.045$) among all the participants, and in Caucasians ($\beta = 0.0075$, $p=0.40$). In the corresponding analyses for hypertension the results were statistically significant among all participants ($\beta=0.1407$, $p=0.0248$) and in Caucasians ($\beta=0.1886$, $p=0.0248$) but not in African-Americans ($\beta=0.2722$, $p=0.1462$) and not in Africans ($\beta=0.1494$, $p=0.1767$).
Conclusion: FOP is significantly, positively associated with SBP and hypertension in the multivariate-adjusted analyses. The association seems to differ by race and ethnicity.

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INTRODUCTION

Oxidative stress is defined as an imbalance between production and elimination of reactive oxygen species (ROS) and other free radicals [1]. ROS, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and organic peroxides, which are generated in the mitochondrial respiratory chain and adversely alter lipids, proteins and DNA [2]. Under hypoxic conditions, the mitochondrial respiratory chain also generates reactive nitrogen species (RNS), which also act as free radicals [3].

Many of the lifestyle and dietary factors are established pro-oxidants [4]. Inhaled tobacco smoke is considered to be one of the pro-oxidants due to high concentrations of ROS and RNS in tar and gas [5]. Dietary fat also exerts pro-oxidant effect through increased lipid peroxidation. Iron adds to oxidative stress by catalyzing ascorbate oxidation and by producing highly reactive hydroxyl radicals via the Haber-Weiss reaction [6].

In order to protect against harmful effects of oxidative stress, organism employs a variety of intrinsic and extrinsic agents including enzymatic antioxidants such as catalase, glutathione peroxidases (GPx), and superoxide dismutase (SOD); chain breaking anti-oxidants (e. g., tocopherols, Vitamin C, carotenoids, uric acid, flavonoids, and glutathione); and metal binding proteins including transferrin and lactoferrin [7]. Under normal conditions, antioxidants outbalance pro-oxidants, but under oxidative conditions, pro-oxidants prevail over antioxidants and for this reason oxidative stress is viewed as an imbalance between pro-oxidants and anti-oxidants [8].

Oxidative stress leads to damage of important biomolecules with potential impact on the cell function. Studies demonstrate that oxidative stress affects the regulation of

signaling pathways, influences synthesis of antioxidant and DNA repair enzymes, and is closely related to cell apoptosis and proliferation. Oxidative stress also induces inflammation and inflammation in-turn increases oxidative stress [8]. The closely intertwined processes of oxidative stress and inflammation are linked to aging and are thought to be responsible for a number of age-related human diseases [9].

One chronic condition that is thought to be both caused by and result in oxidative stress is arterial hypertension. The pathophysiology of hypertension involves functional and structural changes of blood vessels including endothelial damage, smooth muscle dysfunction and alteration of vascular contraction. Oxidative stress participates in all these processes [10]. ROS and RNS are directly involved in the inactivation of the vasodilator $NO^{\cdot}$ molecules, generation of vasoconstrictor lipid peroxidation products, depletion of tetrahydrobiopterin, activation of pro-inflammatory transcription factors and stimulation of growth factor production. Hypertension can also cause oxidative stress by increasing inflammation and accelerating generation of ROS and RNS [11]. This creates a vicious cycle that can lead to progressive deterioration of hypertension.

It is important to point out that much of the mechanistic evidence linking hypertension to oxidative stress comes from in vitro and animal studies. By contrast population-based human studies addressing this issue are relatively rare due to the difficulty of measuring oxidative stress in humans. In order to assess oxidative stress researchers rely on biomarkers. Currently F_2-isoprostanes (F_2IP) are considered to be the "gold standard" oxidative stress markers [12]. F_2IP are the products of non-enzymatic lipid peroxidation that are detectable in plasma and urine. The main disadvantage of F_2IP use in human research is their relative instability. Accurate

measurements of F_2IP require careful handling and rapid processing of samples, which may not always be possible in population-based studies [13]. In addition, F_2IP only reflect one aspect of oxidative stress (lipid peroxidation) and do not provide direct information about free radical-induced damage of other macromolecules such as DNA and protein [12].

A recently proposed alternative to F2IP is fluorescent oxidation products (FOP) [14]. Unlike F2IP, FOP reflects a mixture of oxidation products from lipids, proteins and DNA. Previous studies have shown that FOPs are associated with smoking, coronary heart disease, and chronic kidney disease [14-16]. One study also demonstrated that plasma FOP levels were higher in hypertensive men compared to men with normal blood pressure [14]. However the association between FOP and blood pressure has not been examined among women. Moreover, nearly all participants in the previous study of FOP and hypertension were whites [14]. It is important to point out that the association between oxidative stress and hypertension may vary in whites and blacks. [17-19]. Moreover, this association may be different in African Americans and sub-Saharan Africans the two groups that are often categorized together as "blacks" but are very dissimilar with respect to both genetic and lifestyle-related factors [20]. The purpose of this study is to examine the association between FOP and hypertension in men and women residing in the state of Georgia and representing three racial and ethnic groups: US-born Caucasians, African-Americans and West African immigrants. The secondary objective of this study is to further evaluate the utility of using FOP as oxidative stress biomarkers.

METHODS

Study population

We used the data from the Study of Race, Stress and Hypertension (SRSH), which included three groups of participants: US whites (n=116), African-Americans (n=78) and West Africans (n=79) residing in the state of Georgia. The first two groups were randomly selected among participants in a previously completed cross-sectional study The West Africans were recruited in the Atlanta churches attended by primarily Nigerian or Ghanaian immigrants. The eligible participants were between ages of 24 and 74 and were permanent residents of the State of Georgia. The race/ethnicity of participants was based on self-identification.

Overview of data collection

The data collection for all three groups was conducted using the same study protocol. Recruitment and data collection took place at previously scheduled events (e.g., after church service for Africans or during a community festival for the other two groups). The data and samples were collected in a designated area with study "stations" described below:

1. Informed Consent station – A study team member reviewed the consent forms in detail with the interested participant. The eligibility of the participants was assessed by a team member and recorded on the eligibility form. Once consent and eligibility are authorized, the participant may progress to any of the other stations (2-6).

2. Blood pressure station – Trained and certified staff measured participants' blood according to study protocol. The observer gave the participant an explanation of the

blood pressure procedure while the participant is seated in a quiet room with no change of position or posture. Measured BP three times, and make sure there are 30 seconds between two measurements.

3. Height & weight station – a study team member asked the participant a few questions about their height and weight, and then measures and records information according to study protocols via the Eligibility & Interview form.

4. Blood draw station - Vacutainer-type blood collection tubes were drawn from each participant by a phlebotomist. The blood was putted to one 10 ml green top sodium heparin tube (plasma) and was placed on wet ice and covered to protect the samples from light. All samples were transported to the Emory University Lab at the end of the visit.

5. Questionnaire review station – After signing consent and being screened as eligible, the participant will fill out a Study Questionnaire, which gathers contact, demographic, and essential medical history information as well as more in-depth information on the participant's physical activity, weight history, tobacco and alcohol use, and more in-depth medical and family health information including medication use.

Assessment of hypertension and blood pressure measurement

Systolic and diastolic blood pressures (SBP and DBP) were measured three times by trained staff on blood pressure station described above. We measured the blood pressure in a silent room and chose the proper cuff size according to the circumference of the arm. After recorded the pulse and determined the peak inflation level, we measured systolic and diastolic blood pressure three times and took the average value of three measurements. All participants were also asked if they were taking any BP lowering

medications, and if so they took those medications on the day of data collection. Hypertension was defined as average SBP of 140 mmHg or higher, or the average DBP ≥ 90 mmHg, or self-reported use anti-hypertension drugs.

Measurement of Fluorescent Oxidation Products (FOP)

Plasma samples were drawn into a 10 ml green top sodium heparin tube. All samples were immediately placed on ice and delivered to the laboratory where the blood was processed and centrifuged. Plasma and serum were separated, aliquoted, and frozen at -80 °C for long-term storage.

Fluorescent oxidation products (FOP) were measured according to the method developed by Dillard and Tappel and modified by Shimasaki [21, 22]. Plasma was extracted with ethanol-ether (3/1, v/v) and vigorously mixed on a vortex mixer. The mixed solution was centrifuged for 10 minutes at 3000 rpm, and 1 mL of supernatant was added to cuvettes for spectrofluorometric readings [23]. The actual measurement was expressed as relative fluorescence intensity units per milliliter of plasma at 360 nm excitation and 465 nm emission for all readings. FOP concentrations were calculated against a 1 ppm fluorescent reference standard quinine in 0.1 N Sulfuric Acid.

Assessment of other variables

Physical activity was calculated using the modified Paffenbarger Questionnaire [24]. Vigorous activity included heavy carpentry, moving furniture, digging strenuous sports, jogging, chopping wood, bicycling on hills, swimming laps, aerobics, and other similar tasks. Moderate activity included tasks such as light carpentry, housework,

painting, yard work, household repairs, walking, recreational swimming or skating, bicycling on level ground, golf without a motorized cart, and tennis. We recorded the minutes of vigorous and moderate physical activity, on weekdays and weekends. Based on this information, we calculated the average Metabolic Equivalent of Task (MET) MET-minutes per week and the average MET-minutes per day.

Age, marital status, education level, tobacco and drinking use, NSAID and statins medication history were assessed through the self-report questionnaire we conducted in the Questionnaire review station described above. Body mass index was calculated based on the weight and height we measured on the Height & weight station using formula: $BMI=weight/height^2$ (kg/m^2).

Statistical analysis

The distributions of categorical variables across the three racial/ethnic study groups were examined via cross-tabulation with chi-square tests. Analysis of variance (ANOVA) tests were used to compare distributions of continuous variables. We used linear regression models with log transformation of the dependent variable, FOP, because its distribution was skewed. Simple and multiple linear regression analyses were performed to assess the relation of FOP to SBP, DBP and hypertension. The results of multivariable models were adjusted for body mass index (BMI), physical activity, and age, all used as continuous variables, and for sex (male, female), history of smoking (never or past, current), history of drinking (never or past, current), use of non-steroidal anti-inflammatory drugs (NSAID) and statins (yes, no), marital status (single, married,

divorced or widowed), and education level (above bachelor degree, below bachelor). We examined the same association within each racial/ethnic group. All models were assessed for interactions, collinearity and the goodness-of-fit. A two-sided p-value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC) software package.

RESULTS

Among 273 study participants, 101 (38%) were male and the age ranged from 24 to 74 years. The mean plasma FOP concentration was 0.0422 FIU/ml with a standard deviation (SD) of 0.0191 FIU/ml). Selected characteristics of study participants are shown in Table 1. About one-third (35%) had hypertension with the highest prevalence among African-Americans (50%) and the lowest prevalence among African immigrants (25%). Both SBP and DBP differed significantly ($p<0.01$) across the three study groups with the highest average values among African Americans.

The distributions of behavioral, social and medical factors among study participants are shown in Table 2. A statistical comparison across the three racial/ethnic groups was not performed due large differences in missing data. Overall 40% of the participants had at least a college degree, and 67% were married at the time of the study. The proportions of drinkers ranged between 67% in Caucasians and 22% in African immigrants. The corresponding proportions of current smokers were 28%, 17% and 4% for Caucasians, African-Americans and Africans, respectively. With respect to medication use and physical activity, Caucasians were the group with the highest prevalence of taking statins (19%) and NSAIDs (27%) and the lowest reported weekly MET estimates (434 MET minutes/day).

In crude models, the blood pressure measures and hypertension were not associated with FOP concentrations (Table 3). As about 25% of subjects had missing data, we removed subjects with missing data to set up an alternative crude model aimed at assessing the selection bias attributable to non-response. After removing subjects with missing data, the association between FOP and SBP was statistically significant for

all three groups combined (β=0.0043, p=0.0209). After stratification by race/ethnic groups, only the results of Caucasians remained statistically significant the crude model without missing data (β=0.0068, p=0.0153).

No association was observed between DBP and FOP concentrations in any of the crude models. In the DBP analyses the β-coefficients ranged from -0.0012 among all subjects combined to 0.0043 among Caucasians.

The crude association between hypertension and FOP was only observed among Caucasians (β=0.1773, p=0.0146), but not among other races/ethnic groups or in the total study population. Generally, the regression coefficients of the crude models without missing data were between the crude estimates for all subjects and the adjusted estimate.

In the multivariable regression analysis, there was a positive and statistically significant association between FOP and SBP (β=0.0042, p=0.0448) in all three groups combined. After stratification by racial/ethnic group, the association remained statistically significant only among Caucasians (β=0.0075, p=0.0397), but not among Africans (β=0.0039, p=0.1933), and African-Americans (β=-0.0025, p=0.7226). No multivariable-adjusted associations were observed between DBP and FOP either overall or after stratification by race/ethnicity (Table 3). In the corresponding analyses for hypertension the results were statistically significant among all participants (β=0.1407, p=0.0248) and in Caucasians (β=0.1886, p=0.0248) but not in African-Americans (β=0.2722, p=0.1462) and not in Africans (β=0.1494, p=0.1767). Despite these differences the interaction between race/ethnicity and hypertension was not statistically significant.

DISCUSSION

In this study fluorescent oxidation products (FOPs) were significantly, positively associated with systolic blood pressure and hypertension after adjusting for confounders. Although the interaction was not statistically significant the associations appeared to differ by race/ethnicity.

From the biological perspective, hypertension involves endothelial damage, smooth muscle dysfunction and alteration of vascular contraction, the processes that are also affected by oxidative stress [10]. In addition, hypertension causes oxidative stress by increasing inflammation and accelerating generation of ROS and RNS [11]. Although it is plausible that hypertension is associated with markers of oxidative stress including FOP the exact biological significance of FOPs is still unclear.

There are few human studies investigating the relationship between FOPs and health outcomes in humans. One nested case-control study (n=788), found that plasma FOPs significantly and independently predicted coronary heart disease incidence among men without previous cardiovascular events [16]. In a cross-sectional study among men (n=525), Wu et al reported that FOP positively associated with hypertension [14]. Our results are consistent with the previous study research although the two populations were rather different. The participants in the Wu et al study were mainly Caucasian males, while our study included males and females of different racial and ethnic backgrounds.

In addition to the unique study population and the use of a relatively novel biomarker of oxidative stress a distinguishing feature of this study was the careful collection of blood samples. The samples were placed on ice and immediately processed aliquoted and stored at -80 degrees. Thus the results are likely to be reliable

and not affected by sample degradation.

The main limitation of our study is missing covariates. For this reason it is not clear whether the difference in crude and adjusted results could be explained by confounding or selection bias attributable to removal of subjects with missing data. To analyze this problem, we compared three sets of results: unadjusted associations for all persons with blood pressure and FOP data, similar unadjusted association for persons without any missing covariates and adjusted results after controlling for covariates. Based on our comparison of the three sets of results it appears that only the association between FOP and hypertension for all subjects combined remained reasonably robust regardless of the analyses. Since this study used a cross-sectional design, it is unable to establish the temporal relationship between FOPs and hypertension. Thus longitudinal observational studies are needed to determine if FOP increases in response to hypertension, or vice versa, or if the relation between hypertension and oxidative stress is truly bi-directional

References:

1. Sies, H., *Oxidative stress: from basic research to clinical application*. Am J Med,, 1991. **91**(3C): p. 315-385.
2. Ziech, D., et al., *The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development*. . Chem Biol Interact, 2010. **188**(2): p. 334.
3. Poyton, R.O., K.A. Ball, and P.R. Castello, *Mitochondrial generation of free radicals and hypoxic signaling*. Trends Endocrinol Metab, 2009. **20**(7): p. 332-40.
4. Goodman, M., et al., *Clinical trials of antioxidants as cancer prevention agents: past, present, and future*. Free Radic Biol Med, 2011. **51**(5): p. 1068-84.
5. van der Vaart, H., et al., *Acute effects of cigarette smoke on inflammation and oxidative stress: a review*. Thorax, 2004. **59**(8): p. 713-21.
6. Puntarulo, S., *Iron, oxidative stress and human health*. Mol Aspects Med, 2005. **26**(4-5): p. 299-312.
7. Young, I.S.a.J.V.W., *Antioxidants in health and disease*. J Clin Pathol, , 2001. **54**(3): p. 176-86.
8. Reuter, S., et al., *Oxidative stress, inflammation, and cancer: how are they linked?* Free Radic Biol Med, 2010. **49**(11): p. 1603-16.
9. Devasagayam, T.P., et al., *Free radicals and antioxidants in human health: current status and future prospects*. J Assoc Physicians India, 2004. **52**: p. 794-804.
10. Briones, A.M. and R.M. Touyz, *Oxidative stress and hypertension: current concepts*. Curr Hypertens Rep., 2010. **12**(2): p. 135-42-doi: 10.1007/s11906-010-0100-z.
11. Vaziri, N.D., *Roles of oxidative stress and antioxidant therapy in chronic kidney disease and hypertension*. Curr Opin Nephrol Hypertens., 2004. **13**(1): p. 93-9.
12. Musiek, E.S. and J.D. Morrow, *F2-isoprostanes as markers of oxidant stress: an overview*. Curr Protoc Toxicol., 2005. **Chapter 17**: p. Unit 17.5. doi: 10.1002/0471140856.tx1705s24.
13. Kitano, S., et al., *Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning?* Free Radic Biol Med, , 2005. **38**(6): p. 698-710.
14. Wu, T., et al., *Plasma fluorescent oxidation products as potential markers of oxidative stress for epidemiologic studies*. Am J Epidemiol., 2007. **166**(5): p. 552-60-Epub 2007 Jul 5.
15. Rebholz Cm Fau - Wu, T., et al., *The association of plasma fluorescent oxidation products and chronic kidney disease: a case-control study*. (1421-9670 (Electronic)).
16. Wu, T., et al., *Plasma fluorescent oxidation products: independent predictors of coronary heart disease in men*. Am J Epidemiol., 2007. **166**(5): p. 544-51-Epub 2007 Jul 5.
17. Kalinowski, L., Dobrucki, I. T., Malinski, T., *Race-specific differences in endothelial function: predisposition of African Americans to vascular diseases*. . Circulation, 2004. **109**(21): p. 6.
18. Lopes, H.F., Morrow, J. D., Stojiljkovic, M. P., Goodfriend, T. L., & Egan, B. M.,

Acute hyperlipidemia increases oxidative stress more in African Americans than in white Americans. Am J Hypertens, 2003. **16**: p. 331-336.

19. Watters, J.L., Satia, J. A., & Kupper, L. L., *Correlates of antioxidant nutrients and oxidative DNA damage differ by race in a cross-sectional study of healthy African American and white adults*. Nutr Res, 2008. **28**(9): p. 565-576.
20. Opie, L.H., & Seedat, Y. K., *Hypertension in sub-Saharan African populations*. Circulation, 2005. **112**(23): p. 3562-3568.
21. Dillard CJ, T.A., *Fluorescent products of lipid peroxidation of mitochondria and microsomes*. . Lipids 1971. **6**: p. 715-12.
22. Shimasaki, *Assay of fluorescent lipid peroxidation products*. Methods Enzymol, 1994. **233**: p. 338-46.
23. Wu T, R.N., Roberts LJ 2nd, et al., *Stability of measurements of biomarkers of oxidative stress in blood over 36 hours*. Cancer Epidemiol Biomarkers Prev 2004.
24. Ralph S. Paffenbarger, J., *Measurement of physical activity to assess health effect in free-living populations*. Med Sci Sports Exerc. , 1993. **25**(1): p. 60-70.

Table 1. Characteristics of Study Participants

	Caucasian (N=116)	African American (N=78)	African (N=79)	Total (N=273)	P-value
Age (SD), years	48.00 (12.99)	48.38 (10.30)	42.47 (10.90)	46.47 (11.94)	0.002
Males, N (%)	35 (30%)	38 (52%)	28 (37%)	101 (38%)	0.011
BMI (SD), kg/m^2	29.26 (5.86)	30.59 (6.46)	28.92 (5.05)	29.52 (5.83)	0.170
SBP (SD), mmHg	122.33 (12.37)	127.67 (13.92)	121.10 (15.51)	123.34 (13.97)	0.010
DBP (SD), mmHg	74.75 (7.81)	81.24 (9.90)	73.28 (8.30)	76.00 (9.09)	<0.001
Hypertension, N (%)	37 (32%)	39 (50%)	20 (25%)	96 (35%)	0.003
FOP (SD), FIU/ml	0.0418 (0.0199)	0.0399 (0.0147)	0.0518 (0.0200)	0.0442 (0.0191)	<0.001

Acronyms: BMI=body mass index; FIU=fluorescence intensity units

Table 2. Distributions of behavioral, social and medical factors among study participants

	Caucasian (N=116)	African American (N=78)	African (N=79)	Total (N=273)
Current Drinkers, N (%)	57(67%)	16(36%)	17(22%)	90(43%)
Missing data	32(28%)	34(44%)	1(1%)	67(25%)
Current Smokers, N (%)	32(28%)	13(17%)	3(4%)	48(18%)
Missing data	32(28%)	34(44%)	1(1%)	67(25%)
Taking statins, N (%)	19(16%)	11(14%)	3(4%)	33(12%)
Missing data	32(28%)	34(44%)	0(0%)	66(24%)
Taking NSAID, N (%)	31(27%)	10(12%)	11(14%)	52(20%)
Missing data	32(28%)	34(44%)	0(0%)	66(24%)
Marital status, N (%)				
Never Married	12(10%)	15(19%)	11(14%)	38(14%)
Current in marriage	91(79%)	38(49%)	53(67%)	182(67%)
Divorced or Widowed	12(10%)	23(29%)	15(19%)	50(18%)
Missing data	1(1%)	2(3%)	0(0%)	3(1%)
Education, N (%)				
High school or less	67(58%)	57(73%)	37(47%)	161(59%)
Bachelor or above	48(41%)	21(27%)	41(52%)	110(40%)
Missing Data	1(1%)	0(0%)	1(1%)	2(1%)
Physical Activity				
Average METs - minutes/day	433.95	470.88	597.38	503.72

* MET= Metabolic Equivalent of Task

Table 3. Linear regression analyses of FOP in relation to blood pressure and hypertension

		β Coefficient	Standard Error	R^2	P value
Systolic Blood Pressure					
All participants	**Crude**	0.0025	0.0015	0.0101	0.1052
	Crude (subjects with non-missing data)	0.0043	0.0018	0.0265	0.0209
	Adjusted*	0.0042	0.0021	0.1600	0.0448
Caucasian	**Crude**	0.0024	0.0023	0.0093	0.3033
	Crude (subjects with non-missing data)	0.0068	0.0027	0.0696	0.0153
	Adjusted*	0.0075	0.0036	0.2019	0.0397
African	**Crude**	0.0046	0.0026	0.0409	0.0759
	Crude (subjects with non-missing data)	0.0048	0.0026	0.0442	0.0665
	Adjusted*	0.0039	0.0030	0.1594	0.1933
African American	**Crude**	0.0034	0.0031	0.0178	0.2778
	Crude (subjects with non-missing data)	0.0030	0.0054	0.0084	0.5733
	Adjusted*	-0.0025	0.0070	0.3090	0.7226
Diastolic Blood Pressure					
All participants	**Crude**	-0.0012	0.0024	0.0009	0.6298
	Crude (subjects with non-missing data)	0.0000	0.0031	0.0000	0.9919
	Adjusted*	0.0042	0.0035	0.1479	0.2212
Caucasian	**Crude**	-0.0002	0.0037	0.0000	0.9641
	Crude (subjects with non-missing data)	0.0043	0.0049	0.0096	0.3760
	Adjusted*	0.0067	0.0053	0.1703	0.2088
African	**Crude**	0.0027	0.0049	0.0040	0.5837
	Crude (subjects with non-missing data)	0.0025	0.0049	0.0036	0.6047
	Adjusted*	0.0024	0.0061	0.1370	0.6906
African American	**Crude**	0.0024	0.0044	0.0046	0.5846
	Crude (subjects with non-missing data)	0.0024	0.0079	0.0025	0.7613
	Adjusted*	-0.0052	0.0112	0.3113	0.6482
Hypertension					
All participants	**Crude**	0.0746	0.0447	0.0102	0.0961
	Crude (subjects with non-missing data)	0.1041	0.0534	0.0183	0.0527
	Adjusted*	0.1407	0.0622	0.1782	0.0248
Caucasian	**Crude**	0.0811	0.0617	0.0149	0.1913
	Crude (subjects with non-missing data)	0.1773	0.0710	0.0706	0.0146
	Adjusted*	0.1886	0.0902	0.2016	0.0403
African	**Crude**	0.1655	0.0895	0.0425	0.0683
	Crude (subjects with non-missing data)	0.1623	0.0901	0.0409	0.0757
	Adjusted*	0.1494	0.1090	0.1608	0.1767
African American	**Crude**	0.1224	0.0814	0.0289	0.1366
	Crude (subjects with non-missing data)	0.1172	0.1288	0.0193	0.3680
	Adjusted*	0.2722	0.1824	0.2865	0.1462

* Adjusted for sex, age, race, BMI, marital status, physical activity, education level, NSAID and statin use, smoking, and alcohol drinking.