**Inflammatory Obesity Phenotypes, Gender Effects, and Subclinical Atherosclerosis in African Americans**

**The Jackson Heart Study**

Albert Lin, Mary E. Lacy, Charles Eaton, Adolfo Correa, Wen-Chih Wu

**Objective**—Reasons for variations in atherosclerotic burden among individuals with similar levels of obesity are poorly understood, especially in African Americans. This study examines whether high-sensitivity C-reactive protein (hsCRP) is useful for discriminating between benign and high-risk obesity phenotypes for subclinical atherosclerosis in African Americans.

**Approach and Results**—Participants from the Jackson Heart Study (n=4682) were stratified into 4 phenotypes based on the presence of obesity (body mass index (BMI) ≥30 or body mass index 25–30 with waist circumference >102 cm in men and >88 cm in women) and inflammation by hsCRP ≥2 mg/L. Using multivariate regression models, we conducted cross-sectional analyses of the association between inflammatory obesity phenotypes and subclinical atherosclerosis determined by carotid intima–media thickness or coronary artery calcium scores. Sex-specific analyses were conducted given significant interaction for gender (P=0.03). The prevalence of obesity or equivalent was 65%, of which 30% did not have inflammation. Conversely, 37% of nonobese individuals had inflammation. Among nonobese men, hsCRP ≥2 mg/L identified a subset of individuals with higher carotid intima–media thickness (adjusted mean difference =0.05, 95% confidence interval 0.02, 0.08 mm) compared with their noninflammatory counterparts. Among obese men, hsCRP <2 mg/L identified a subset of individuals with lower coronary artery calcium compared with their inflammatory counterparts. Among women, associations between hsCRP and carotid intima–media thickness or coronary artery calcium were not found.

**Conclusions**—In the largest African American population-based cohort to date, hsCRP was useful in identifying a subset of nonobese men with higher carotid intima–media thickness, but not in women. hsCRP did not identify a subset of obese individuals with less subclinical atherosclerosis. *(Arterioscler Thromb Vasc Biol. 2016;36:2431-2438. DOI: 10.1161/ATVBAHA.116.307728.)*

**Key Words:** carotid intima–media thickness • coronary artery calcium • gender • high-sensitivity C-reactive protein • inflammation • obesity • subclinical atherosclerosis

Among African American (AA) adults, 76% are overweight or obese and 19% have diabetes mellitus, rates that are substantially higher than in the general population (66% and 11%, respectively).1,2 Both excess obesity and diabetes mellitus are major risk factors for cardiovascular disease,1,2 and they themselves are major factors of which, if better understood, may translate into more successful prevention efforts to mitigate disparities in the prevalence of diabetes mellitus and cardiovascular disease in AAs.3 Data from mostly White cohorts suggest that the presence of inflammation is one factor that could be helpful in discriminating between the benign and high-risk phenotypes across body mass index (BMI) and waist circumference–based classifications of obesity.4–9 High-sensitivity C-reactive protein (hsCRP) is an easy-to-measure and inexpensive inflammatory biomarker that has been associated with obesity and atherosclerotic disease burden.4 Therefore, it is possible that inflammation, or its absence as measured by hsCRP, could be helpful in explaining the variation in the subclinical atherosclerosis, if any, associated with obesity among AAs by identifying a subset of obese individuals without an increased atherosclerotic burden (benign obesity) compared with nonobese individuals. It may also help to identify, if it exists, a nonobese subpopulation that has a higher burden of subclinical atherosclerosis compared with their noninflammatory counterparts. This is especially important because median hsCRP levels are known to be higher in AAs than in whites; therefore, hsCRP in AAs have the potential...
to identify a larger at-risk population for subsequent cardiovascular events because of increased atherosclerotic burden.9

Because there is a paucity of information that characterizes the interaction between obesity and inflammation as they relate to subclinical atherosclerosis in the AA population, we studied whether the inflammatory obesity phenotype was related to subclinical carotid or coronary atherosclerosis, as measured by carotid intima–media thickness (cIMT) and coronary artery calcium (CAC) scores, respectively. We performed this analysis using the largest population-based observational cohort of AAs to date, the Jackson Heart Study (JHS). We hypothesize that hsCRP levels will identify a subset of nonobese AA adults with a higher atherosclerotic burden related to inflammation and another subset of obese AA adults without inflammation with a lower burden of atherosclerosis.

### Results

#### Baseline Characteristics

Of 4682 individuals without cerebrovascular disease at baseline, 65% of them met study criteria for obesity based on BMI and waist circumference. We found 37% of nonobese individuals with significant inflammation by hsCRP ≥2 mg/L criteria, while 30% of obese participants did not have significant

| Table 1. Baseline Characteristics by Inflammatory Obesity Phenotypes |
|------------------|------------------|------------------|------------------|------------------|
| Characteristic   | Overall (n=4682) | Nonobese*       | Obese*           | P Values: Overall and for Group 1 vs 2, 3, 4 |
| Male, %          | 1720 (36.7)       | 601 (58.0)       | 313 (52.3)       | 356 (39.3)       | 450 (21.0)       | a, b, c, d |
| Age, mean (SD)   | 54.52 (12.72)     | 52.70 (13.60)    | 55.88 (12.68)    | 55.36 (12.74)    | 54.68 (12.17)    | a, b, c, d |
| Height, cm, mean (SD) | 169.00 (9.30) | 171.58 (8.49)    | 170.09 (9.39)    | 170.21 (9.73)    | 166.94 (8.52)    | a, b, c, d |
| Weight, kg, mean (SD) | 90.37 (21.27) | 74.68 (12.00)    | 73.98 (11.34)    | 95.12 (18.04)    | 100.52 (20.96)   | a, c, d |
| Waist, cm, mean (SD) | 100.46 (16.10) | 86.30 (8.89)     | 87.07 (8.53)     | 104.59 (11.68)   | 109.31 (14.75)   | a, c, d |
| BMI, kg/m², mean (SD) | 31.66 (7.68) | 0.82 (0.52)      | 6.18 (9.11)      | 1.06 (0.53)      | 8.33 (8.89)      | a, b, c, d |
| Alcohol, %       | 2178 (46.7)       | 569 (55.1)       | 318 (53.4)       | 382 (42.4)       | 909 (42.6)       | a, c, d |

P values calculated using χ² and 1-way ANOVA with Scheffe test comparing nonobese and hs-CRP<2 to each of other 3 groups: a=overall (P<0.05), b=group 1 vs 2 (P<0.025), c=group 1 vs 3 (P<0.025), d=group 1 vs 4 (P<0.025). Alcohol use, any drinking in past 12 mo; Smoking, cigarette smoking only; Hypertension, SBP ≥140 mm Hg, DBP ≥90 mm Hg, and use of blood pressure–lowering medications in 2 wk before baseline visit; Hypercholesterolemia, fasting total cholesterol ≥240 mg/dL, fasting LDL cholesterol ≥160 mg/dL, and use of cholesterol-lowering medications in 2 wk before baseline visit; Coronary artery disease: history of physician-diagnosed myocardial infarction, coronary revascularization procedure, or abnormal stress test; Peripheral vascular disease: peripheral vascular surgery, angiography, or revascularization; Diabetes mellitus: Fasting glucose ≥126 mg/dL, HbA1c ≥6.5%, and use of antidiabetic medications in 2 wk before baseline visit. ANOVA indicates analysis of variance; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; and NHLBI, National Heart and Lung and Blood Institute.

*Obesity defined based on NHLBI recommendations, using a combination of BMI and gender-specific waist circumference thresholds.

### Materials and Methods

#### Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Participants from the Jackson Heart Study (n=4682) were stratified into 4 phenotypes based on the presence of NHLBI definition of obesity or obesity-equivalent (BMI ≥30 or BMI 25–30 with waist circumference >102 cm in men and >88 cm in women) and inflammation by hsCRP ≥2 mg/L. Alternatively, we conducted sensitivity analyses using a cut-point of the upper quartile of gender-obesity–specific hsCRP levels to define inflammation. Using multivariate regression models, we conducted cross-sectional analyses of the association between inflammatory obesity phenotypes and subclinical atherosclerosis determined by carotid intima-media thickness (cIMT) or coronary artery calcium scores (CAC).
inflammation. The baseline characteristics of the overall and the 4 inflammatory obesity phenotypes (groups 1 through 4) of AA individuals are summarized in Table 1. Compared with the other 3 groups of participants with either obesity, inflammation, or both, individuals from group 1 were more likely to be younger, male, and less likely to have a history of hypertension. Among the nonobese individuals, those with hsCRP ≥2 mg/L (group 2) were more likely to be current smokers. Compared with group 1, obese individuals, with or without inflammation, tended to have a higher prevalence of diabetes mellitus and hypercholesterolemia, but have a lower prevalence of alcohol use.

**BMI and hsCRP**

BMI and hsCRP values were significantly correlated in the overall population (both men and women; Table 2). Figure 1 showed hsCRP levels to be similar between men and women across inflammatory obesity phenotypes except for group 4 (obese and inflammatory), where women have higher hsCRP levels (mean hsCRP for women =8.77 and for men =6.67 mg/L; \( P<0.01 \)). In contrast, men had greater average waist circumference and BMI values compared with women, with the exception of group 4, where women had a significantly higher BMI (\( P<0.05 \); in Figures III and IV in the online-only Data Supplement).

**Obesity Phenotypes and cIMT**

Women had lower mean cIMT values than men across all obesity phenotypes (\( P \) value <0.05 for all comparisons), with the exception of group 3 (\( P=0.30 \); Figure 2). In addition, mean cIMT values were significantly different across inflammatory obesity phenotypes (\( P<0.01 \)), with the lowest cIMT values found in individuals of group 1. Adjusted analyses in Table 3 showed obese men and women, irrespective of hsCRP levels (groups 3 and 4), to have significantly higher cIMT values compared with group 1 (referent). However, the association between inflammatory obesity phenotypes and cIMT differed by gender (\( P=0.03 \) for interaction). Elevated hsCRP values identified a subgroup of nonobese men (group 2), but not women, with higher cIMT values compared with group 1. Further adjustment by BMI and waist circumference did not significantly change these results. This relationship was maintained for low-risk individuals, such as those without prior coronary artery or peripheral vascular disease. Use of alternative hsCRP thresholds to define inflammation derived from gender- and obesity-specific cut points (baseline characteristics of the obesity phenotypes reclassified are shown in Table I in the online-only Data Supplement) did not qualitatively change the results (Table II in the online-only Data Supplement).

**Obesity Phenotypes and CAC**

A total of 2097 individuals were included in the CAC analysis (Figure II in the online-only Data Supplement). The changes of hsCRP, BMI, and waist circumference between visits 1 and 2 among the subsample of 2097 individuals included in the CAC analysis are shown in Table III in the online-only Data Supplement. Between visits 1 and 2, significant increases in BMI and waist circumference occurred for both men and women, whereas only men had significant increases in hsCRP levels. The baseline characteristics of this subsample (Table IV in the online-only Data Supplement) were similar to those of the overall population described for Table 1. Of these individuals, 42% had coronary atherosclerosis (CAC>0). Similar to cIMT results, women had a lower prevalence of coronary atherosclerosis (38.3% for women versus 47.8% for men; \( P<0.01 \)) and lower mean CAC scores (81.17, 95% confidence interval 68.44, 93.90 in women versus 156.12, 95% confidence interval 122.12, 190.13 in men; \( P<0.01 \)) than men. In men, mean CAC scores were significantly different across the 4 inflammatory obesity phenotypes (\( P<0.01 \)), whereas mean CAC scores were similar among the 4 obesity phenotypes in women (\( P=0.23 \); Figure 3).

Gender-specific adjusted analyses in Table 4 showed that elevated hsCRP values identified a subgroup of obese AA men, but not women, with an increased prevalence of coronary atherosclerosis (prevalence odds ratio for binary CAC =1.36, 95% confidence interval 1.14, 1.63; prevalence odds ratio for multincategorical CAC =1.79, 95% confidence

---

**Table 2. Correlation Matrix Between BMI, hs-CRP, and Atherosclerosis**

<table>
<thead>
<tr>
<th>CAC</th>
<th>hs-CRP</th>
<th>cIMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (n=4682)</td>
<td>...</td>
<td>0.01</td>
</tr>
<tr>
<td>Male (n=1720)</td>
<td>...</td>
<td>0.08*</td>
</tr>
<tr>
<td>Female (n=2962)</td>
<td>...</td>
<td>0.01</td>
</tr>
<tr>
<td>CAC (n=2097)</td>
<td>hs-CRP</td>
<td>CAC</td>
</tr>
<tr>
<td>Overall (n=2097)</td>
<td>...</td>
<td>0.02</td>
</tr>
<tr>
<td>Male (n=748)</td>
<td>...</td>
<td>0.07</td>
</tr>
<tr>
<td>Female (n=1349)</td>
<td>...</td>
<td>0.04</td>
</tr>
</tbody>
</table>

CAC values are log-transformed to log (CAC+1) for analysis. Correlations (indicated by *) are significant at \( P<0.05 \). BMI indicates body mass index; CAC, coronary artery calcium; cIMT, carotid intima-media thickness; and hs-CRP, high-sensitivity C-reactive protein.
interval 1.20, 2.66). In contrast to cIMT findings, hsCRP did not identify a higher risk phenotype among the nonobese groups (group 1 versus 2). No significant difference was noted after additional adjustment for BMI change, hsCRP change, and change in waist circumference that occurred between visits 1 and 2. When using gender- and obesity-specific quartiles of hsCRP to restratify the obesity phenotypes, results were not qualitatively different, except that the associations were slightly enhanced in the multicategorical CAC analyses for men (Table V in the online-only Data Supplement).

**Discussion**

In the largest AA population-based cohort to date, free of cerebrovascular disease at baseline, we found that 30% of obese participants did not have an hsCRP ≥2 mg/L, whereas 37% of nonobese individuals had an hsCRP ≥2 mg/L. For women, hsCRP did not discriminate the burden of either carotid or coronary atherosclerosis. In contrast, among men, hsCRP ≥2 mg/L identified a subset of nonobese individuals with higher burden of carotid atherosclerosis. In presence of obesity, hsCRP <2 mg/L identified a subset of men with lower burden of coronary atherosclerosis. Despite this last finding, benign obesity did not exist because all phenotypes of obesity were associated with higher carotid atherosclerotic burden in both men and women irrespective of inflammation. Thus, hsCRP utility seems to primarily identify a subgroup of nonobese men with higher burden of carotid atherosclerosis.

Although previous studies have shown men to have higher cIMT than women, to our knowledge, this is the first study that shows gender differences in the association between inflammation and atherosclerosis, where hsCRP ≥2 mg/L was related to higher cIMT in nonobese men but not in women. Results from Multiethnic Study of Atherosclerosis (MESA; 28% AA) and Dallas Heart cohort (47% AA)
demonstrated that a high hsCRP value was useful in identifying an at-risk group of nonobese individuals with higher prevalence of subclinical atherosclerosis but the authors did not find a significant gender effect on the outcomes. However, in an analysis of 52 prospective studies ($n=246\ 669$) to predict cardiovascular events, the investigators found a significant gender heterogeneity in the association between hsCRP and future cardiovascular events, where the association was stronger in men than in women.14 The reason for such gender disparity in the association between obesity, inflammation, and atherosclerosis is unknown, particularly in AAs. It is possible that the lack of association between hsCRP and atherosclerosis in women relates to physiologically higher hsCRP levels found in women, unrelated to inflammation.15 It has been postulated that a portion of hsCRP production is related to peripheral estrogen levels, which may affect liver production of hsCRP, thus, independent of systemic inflammation and other inflammatory markers, such as interleukin-6.16,17 These studies have shown that these estrogen-induced changes in hsCRP are not associated with angiographic progression of atherosclerosis17,18 and may explain the lack of utility hsCRP to identify women with higher atherosclerosis burden in our study. However, the overall lower prevalence of either significant carotid or coronary atherosclerosis in AA women across all obesity phenotypes compared with men (Tables 3 and 4) may also have reduced our power to detect small differences in atherosclerosis within obesity phenotypes for women. This lower prevalence of atherosclerosis in women is not unique to the JHS cohort and has also been shown in Dallas Heart Study and MESA cohorts.17,18

Although we initially used hsCRP $\geq 2$ mg/L to define inflammation, it is also possible that distinct thresholds for hsCRP, thus, independent of systemic inflammation and other inflammatory markers, such as interleukin-6.16,17 These studies have shown that these estrogen-induced changes in hsCRP are not associated with angiographic progression of atherosclerosis17,18 and may explain the lack of utility hsCRP to identify women with higher atherosclerosis burden in our study. However, the overall lower prevalence of either significant carotid or coronary atherosclerosis in AA women across all obesity phenotypes compared with men (Tables 3 and 4) may also have reduced our power to detect small differences in atherosclerosis within obesity phenotypes for women. This lower prevalence of atherosclerosis in women is not unique to the JHS cohort and has also been shown in Dallas Heart Study and MESA cohorts.17,18

![Figure 3](image-url)
BMI and hsCRP are needed to define a relationship between obesity phenotypes and atherosclerosis in AA women. In our study, gender- and obesity-specific thresholds (upper quartile) for hsCRP were much higher in obese women (hsCRP ≥ 7.83 mg/L) than in obese men (hsCRP ≥ 4.68 mg/L). When gender- and BMI-specific tertiles of hsCRP were used in the Dallas Heart cohort, an association between hsCRP and coronary atherosclerosis was found among obese women, but with a cutoff for hsCRP set at ≥ 9.9 mg/L.13 However, gender- and BMI-specific thresholds for hsCRP in our study did not significantly change our results. The discrepant findings between our cohort and the Dallas Heart cohort may be related to racial differences in the BMI/waist circumference-based cutoffs for obesity and that different level of hsCRP are elicited in response to the same anthropometric definition of obesity. For example, an hsCRP cutoff of 9.9 mg/L used in Dallas Heart Study would correspond to the 86th percentile of the women in our JHS cohort. Given that studies have shown that ethnicity modifies mean hsCRP levels in women19 and additional differences in the estrogen profile may exist across ethnicities,20 future studies are needed to further elucidate the adequate hsCRP cutoffs, if it exists, for identifying atherosclerosis in obese AA women.

It is also possible that hsCRP may not be the best inflammatory marker for women. In the Nurses’ Health Study and the Health Professionals Follow-up Study, elevated plasma levels of soluble tumor necrosis factor-α receptors (sTNF-R1 and sTNF-R2) were related to an increased risk of future coronary heart disease among women, but not men.21 However, these associations were no longer significant after adjustment for BMI, diabetes mellitus, and hypertension. The only biomarker that remained significant was hsCRP for both men and women. Fibrinogen is an alternative inflammatory biomarker that could be considered for phenotypic recognition in AA women. Although fibrinogen also seems to have gender differences similar to hsCRP,14 it seems to have a higher concentration among AA women22 and was significantly associated with cIMT among AA women.23 Interleukin-6 is another inflammatory marker associated with CAC and significant coronary stenosis in AA adults.24 Compared with hsCRP, interleukin-6 has a stronger association with both cIMT and CAC24,25 and subclinical cerebrovascular disease.26 However, there was no evidence of gender-specific effects of interleukin-6 and atherosclerosis.

Our study failed to show the existence of a benign obesity phenotype because obesity was associated with higher cIMT.

<table>
<thead>
<tr>
<th>Table 4. Association Between Inflammatory Obesity Phenotype and Subclinical Atherosclerosis (CAC Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAC &gt;0, n (%)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>Nonobese</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Obese</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Overall P value‡</td>
</tr>
</tbody>
</table>

Gender-specific analyses

| Men |  |
| Nonobese | hs-CRP <2 mg/L | 112 (39.86%) | Ref | Ref |
| | hs-CRP ≥2 mg/L | 72 (53.33%) | 1.48 (0.97, 2.26) | 1.17 (0.95, 1.44) |
| Obese | hs-CRP <2 mg/L | 77 (45.83%) | 1.42 (0.95, 2.11) | NS | 1.09 (0.89, 1.34) | § |
| | hs-CRP ≥2 mg/L | 97 (59.15%) | 1.79 (1.20, 2.66) | 1.36 (1.14, 1.63) |
| Overall P value‡ | <0.01 |

| Women |  |
| Nonobese | hs-CRP <2 mg/L | 69 (33.17%) | Ref | Ref |
| | hs-CRP ≥2 mg/L | 47 (33.81%) | 0.82 (0.50, 1.34) | 0.88 (0.68, 1.14) |
| Obese | hs-CRP <2 mg/L | 102 (41.30%) | 0.99 (0.66, 1.50) | NS | 0.96 (0.77, 1.19) | NS |
| | hs-CRP ≥2 mg/L | 298 (39.47%) | 1.06 (0.74, 1.51) | 1.07 (0.89, 1.29) |
| Overall P value‡ | 0.07 |

Models adjusted for age, smoking history, alcohol use, hypertension, hypercholesterolemia, cerebrovascular disease/stroke, peripheral vascular disease, and diabetes mellitus. CAC indicates coronary artery calcium; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; NS, non-significant; and POR, prevalence odds ratios.

*Ordinal CAC: categorized as CAC = 0; CAC = 1–10; CAC = 11–100; CAC= 101–300; CAC >300.
†Binary CAC: categorized as CAC>0 vs CAC=0.
‡Compared using transformed values of log (CAC +1).
§Subgroup analysis comparing hs-CRP <2 mg/L and hs-CRP ≥2 mg/L among the obese; P value significant at P<0.025.
in both men and women irrespective of hsCRP levels. Similar results were found in MESA where obesity was associated with coronary atherosclerosis in both men and women with and without elevated hsCRP.\textsuperscript{13} We also noticed that the association between risk factor (in our case obesity and hsCRP) and atherosclerosis appeared to vary by vascular territory, a phenomenon well described in the past. Examples of such was in the Framingham Heart Study, which demonstrated that the excess risk attributed to hypertension is higher for stroke (3.8x in men and 2.6x in women) than for coronary disease (≈2 times for men and women).\textsuperscript{17} An excess risk that also varied by gender. Moreover, in 1632 participants from MESA, HOMA-IR (the Homeostatic Model Assessment of Insulin Resistance) was associated with the presence of CAC, but not abdominal or thoracic aortic calcium,\textsuperscript{28} further supporting distinct mechanisms that may lead to atherosclerosis in different vascular territories. It is theorized that different vascular territories with different luminal diameters have distinct hemodynamic stresses that may predispose them to unique mechanisms of atherosclerosis,\textsuperscript{29–31} mechanisms and risk factors that vary by gender and ethnicity.\textsuperscript{32–34} When the effect of inflammation was studied as a risk factor, it has been demonstrated to be dependent on vascular territory, gender, and race.\textsuperscript{13,31} Example of such distinct correspondence between risk factor and vascular territory was also present in the men portion of our study cohort, where hsCRP but not BMI was significantly correlated to cIMT, whereas BMI but not hsCRP was significantly correlated to CAC (Table 2).

The study has several limitations. The study is observational, and the analyses are cross-sectional; therefore, we cannot determine temporality or causality of the relationships found. Although we noticed a similar trend in identifying nonobese men at risk for subclinical disease in both cIMT and CAC, it was not significant for CAC. This may be related to a reduction in power given the attrition between visit 1 and visit 2. It is also possible that our exclusion of individuals with symptomatic vascular disease has selected out the highest risk population for atherosclerosis and, therefore, underestimated the relationship between inflammatory obesity and atherosclerosis. But, this type of design is useful to determine the additive value of biomarker phenotyping in the risk stratification of seemingly asymptomatic patients who could be at risk for future cardiovascular events related to the increased atherosclerosis burden. Although we used the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) cutoff of hsCRP ≥2 mg/L to discriminate between high and low inflammation burden, we also used gender- and obesity-specific cutoffs as sensitivity analyses given recent studies that have found higher hsCRP cutoffs may provide higher specificity in the women and the obese.\textsuperscript{15,16} Although these radiographic markers of atherosclerosis are correlated with traditional CV risk factors\textsuperscript{36,37} and incident CVD,\textsuperscript{38–41} they cannot directly be extrapolated to cardiovascular outcomes. Further longitudinal analysis will be needed to clarify the association between hsCRP, in conjunction with other biomarkers, to cardiovascular events across gender-specific obesity phenotypes in AAs.

In conclusion, in the largest AA population-based cohort to date, hsCRP ≥2 mg/L was useful in identifying a subset of nonobese men with higher cIMT, but not in women. hsCRP <2 mg/L did not identify a subset of obese AAs with less subclinical atherosclerosis. This would suggest alternative hsCRP cutoffs or other biomarkers more suitable to the female AA population that remain unexplored.

Sources of Funding
This material is based on work supported by the Department of Veterans Affairs, Veterans Health Administration. The Jackson Heart Study is supported by contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, and HHSN268201300050C from the National Heart, Lung, and Blood Institute and the National Institute on Minority Health and Health Disparities. The Providence VA Medical Center provided space and salary support for Dr Wu and Ms Lacy to conduct this research project.

Disclosures
None.

References
Obese individuals, regardless of gender or hsCRP levels, were associated with elevated burden of carotid atherosclerosis compared with nonobese individuals. hsCRP did not discriminate the burden of either carotid or coronary atherosclerosis among women with similar obesity status. Inflammation between insulin resistance and vascular calcification in coronary arteriosclerotic cardiovascular disease (the Rancho Bernardo Study). Ong KL, McClelland RL, D’Agostino RB Sr, Wilson PW, Kannel WB, Levy D. Carotid artery intima-media thickness, plaque and Framingham cardiovascular disease. Circulation. 1999;100:713–716.


Inflammatory Obesity Phenotypes, Gender Effects, and Subclinical Atherosclerosis in African Americans: The Jackson Heart Study
Albert Lin, Mary E. Lacy, Charles Eaton, Adolfo Correa and Wen-Chih Wu

Arterioscler Thromb Vasc Biol. 2016;36:2431-2438; originally published online November 17, 2016;
doi: 10.1161/ATVBAHA.116.307728

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/36/12/2431

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2016/11/22/ATVBAHA.116.307728.DC1
http://atvb.ahajournals.org/content/suppl/2016/11/22/ATVBAHA.116.307728.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Study Sample - the Jackson Heart Study (JHS)

The JHS is a longitudinal, population-based observational cohort that was initiated in 2000 to prospectively investigate the epidemiology and determinants of cardiovascular disease in AAs. Details of the study design and recruitment have been previously published.1 The original JHS cohort is comprised of 5,301 African American participants between 21 and 94 years of age enrolled between September 2000 and March 2004 from the Jackson, Mississippi metropolitan area.

Of the 5,301 participants, we excluded 245 participants with prior cerebrovascular disease from the main analysis of subclinical carotid atherosclerosis. Prior cerebrovascular disease was defined as self-reported or prior physician diagnosis of stroke, angioplasty in the arteries of the neck, or carotid endarterectomy. Additional exclusions were 105 participants who were missing obesity or inflammation assessment and 269 who did not have a baseline carotid ultrasound for measurement of cIMT, resulting in a final study population of 4,682 participants (Supplementary Figure I). Excluded participants were not significantly different from those who were included in the analysis in terms of gender, baseline age, smoking history, waist circumference, weight, use of cholesterol or blood pressure-lowering medications, diabetes status, or prior vascular disease or coronary artery disease.

Definition of obesity and inflammation

Obesity was defined using a combination of BMI and waist circumference as outlined in clinical guidelines established by the National Institute of Health (NIH): BMI ≥30 kg/m² or for participants with a BMI between 25 kg/m² and 30 kg/m², a waist circumference >102 cm for men and >88 cm for women (high-risk overweight or obesity-equivalent). Inflammation was defined as a serum hsCRP level ≥2 mg/L, per criteria used in the JUPITER statin trial.3 As a sensitivity analysis, we also examined population-specific thresholds for hsCRP, utilizing the top 75th percentile of the sex and obesity-specific distribution of hsCRP. Levels of hsCRP were measured using immunoturbidimetric CRP-Latex assay from Kamiya Biomedical Company following manufacturer's high-sensitivity protocol. The inter-assay coefficients of variation on control samples repeated in each assay were 4.5% and 4.4% at CRP concentrations of 0.45 mg/L and 1.56 mg/L respectively. The reliability coefficient for masked quality control replicates was 0.95 for the CRP assay.4 Any of the duplicates that were not within three SDs of the assay were rerun.

Subclinical Atherosclerosis

Primary outcome – carotid intima media thickness (cIMT): At Visit 1 (2000-2004), subclinical carotid atherosclerosis was assessed in participants using carotid ultrasound to measure cIMT. Carotid ultrasonography was performed based on the method of Pignoli et al. modified for the ARIC study.4 A 7.5-MHz rectangular transducer with linear array was used to identify landmarks and survey for any stenotic lesions. Subsequently, the protocol scan was conducted in the longitudinal view to assess three key segments of the right and left carotid arteries: the distal straight 1-cm portion of the common carotid artery; the 1-cm portion of the bifurcation distal to the tip of the flow divider; and the 1-cm portion of the internal carotid artery immediately proximal to the tip of the flow divider. All images were recorded to videocassettes and shipped to a single reading-center for interpretation. Ongoing quality assurance program assessed intra-
and inter-technician repeatability and temporal drift, and includes visual review of suspect arterial dimensions. For this analysis, the cIMT variable represented a maximum likelihood estimate of the average far wall cIMT at the optimal angle of interrogation across the right and left common, bifurcation and internal carotid arteries, expressed in millimeters.

Secondary outcome – coronary artery calcium (CAC): At Visit 2 (2005-2008), subclinical coronary atherosclerosis was assessed using computed tomography (CT) scans to measure CAC. Participants underwent CT scan of their heart and other regions. The details of the CT acquisition and processing protocol can be found in JHS Manual 8 at: https://jacksonheartstudy.org/jhsinfo/Portals/0/pdf/manuals2/Manual%208_CT_01-30-2007%20(1).pdf

After acquisition, images were processed by experienced technologists according to standardized protocols to quantify the amount of CAC using the Agatston score on a TeraRecon Aquarius Workstation (TeraRecon, San Mateo, CA). The reproducibility for coronary calcium scores was excellent at 0.99.

Study covariates

For the main analysis of cIMT, all anthropometric and clinical covariates were taken at baseline examination (Visit 1), between 2000 and 2004. Given the main outcome of subclinical carotid atherosclerosis, covariates selected for confounding adjustment in the multivariable regression models were age, sex, smoking history, alcohol use, peripheral vascular disease, coronary artery disease, hypertension, hypercholesterolemia and diabetes, based on the National Cholesterol Education Program - ATP3 criteria, which contained similar risk factors as the ACC/AHA pooled cohort equations for assessing atherosclerotic cardiovascular disease risk.

To this effect, peripheral vascular disease was defined as having prior vascular surgery, angiography, or revascularization. Coronary artery disease was defined as prior history of physician diagnosed myocardial infarction, of coronary revascularization procedure (percutaneous transluminal angioplasty or bypass surgery), or of abnormal stress test. Hypertension status was defined as blood pressure >140/90 mmHg and/or use of blood pressure lowering medication (actual or self-reported) within 2 weeks prior to the clinic visit. Hypercholesterolemia status was defined as high total cholesterol (≥240 mg/dl) or high LDL cholesterol (≥160 mg/dl) and/or use of cholesterol lowering medications (actual or self-reported) within 2 weeks prior to clinic visit. Diabetes mellitus was defined by one of the following: self-report of a doctor diagnosis, use of anti-diabetic medication, fasting plasma glucose ≥126 mg/dl [7.0 mmol/l] or hemoglobin A1c level ≥ 6.5%. Fasting blood samples were collected according to standardized procedures, and the assessments of plasma glucose and lipids were processed at the Central Laboratory (University of Minnesota). Fasting serum total cholesterol and high-density lipoprotein cholesterol and triglyceride concentrations were assessed with Roche enzymatic methods using a Cobras centrifuge analyzer (Hoffman-La Roche, Inc., Nutley, New Jersey), with the laboratory certified by the Lipid Standardization Program of the Centers for Disease Control and Prevention and the National Heart, Lung, and Blood Institute. Alcohol and smoking were defined by subject self-report; alcohol use was defined as any consumption of alcoholic beverages in the prior 12 months. Participants’ smoking status was categorized as current, former or never based on cigarette use only.

Statistical Analysis
We determined the correlation between BMI and hsCRP with cIMT and CAC, and tested for the multiplicative interaction of BMI*hsCRP on our primary outcome of cIMT. Given significant interaction of BMI*CRP on cIMT (p=0.003), we proceeded our analyses with obesity phenotypes stratified by inflammation. Based on the NIH criteria for obesity/obesity-equivalent and an hsCRP cutoff of 2mg/L per JUPITER3 at baseline, we stratified our population into four mutually-exclusive inflammatory obesity phenotypes:

**Group 1:** Non-inflammatory as defined by hsCRP <2 mg/L AND non-obese as defined by a BMI<25 kg/m² or BMI between 25-30 kg/m² and waist circumference <88 cm in women and <102cm in men.

**Group 2:** Inflammatory with hsCRP ≥2 mg/L AND non-obese as defined by criteria above.

**Group 3:** Non-inflammatory AND obese as defined by BMI ≥30 kg/m² or BMI between 25-30 kg/m² and waist circumference ≥88 cm in women and ≥102 cm in men.

**Group 4:** Inflammatory AND obese as defined by criteria above.

We examined and characterized the distribution of baseline covariates and subclinical atherosclerosis across the 4 groups using frequencies and proportions for categorical covariates, and means and standard deviations for continuous covariates. Variables were tested for equality of variance and normality. Log-transformation of variables was performed when appropriate. Chi square tests and one-way ANOVAs were used to examine differences in proportions and means, respectively, across the four phenotypes. Post-hoc analyses for comparisons on baseline characteristics were also conducted within-obesity subgroups with and without inflammation using Scheffe’s test.

**Primary outcome – cIMT (n = 4,682 participants):** We calculated the mean cIMT value and its corresponding 95% confidence limits within each obesity phenotype and used one-way ANOVAs to examine differences in means across the four groups. cIMT followed an approximately normal distribution and was therefore examined as a continuous measure. Multivariable linear regression models were used to examine the cross-sectional association between the four inflammatory obesity phenotypes and cIMT at Visit 1. Regression models were adjusted for age, sex, smoking history, alcohol use, peripheral vascular disease or coronary artery disease, hypertension, hypercholesterolemia and diabetes. Additional adjustment by BMI and waist circumference was performed to assess for potential residual confounding from the obesity phenotype classifications. A formal test for multiplicative statistical interaction was conducted to examine the potential for effect modification by gender with gender-specific analyses conducted when indicated. Sensitivity analyses were also conducted among those participants with no prior history of coronary artery or peripheral vascular diseases.

As a secondary analysis, we re-classified participants utilizing sex- and obesity-specific thresholds for hsCRP, since hsCRP levels varied by gender and obesity status, to examine whether distinct inflammation thresholds alter the results. As such, the 75th percentile cutoff for hsCRP by sex and obesity-specific strata were:

1. Non-obese Men: 2.60 mg/L and non-obese Women: 3.40 mg/L;
2. Obese Men: 4.68 mg/L and obese Women: 7.83 mg/L
Secondary outcome: We repeated the above analyses using the obesity phenotypes created at baseline and using CAC measures from Visit 2 as the outcome (2005-2008). Due to potential variability in obesity and inflammation status over the 5 year follow-up window, BMI, waist circumference and hsCRP measurements taken at Visit 2 were used to calculate change in BMI, waist circumference and hsCRP from baseline, which were included in the CAC multivariable regression analyses to account for potential variability over time. Of the 5,301 participants enrolled at baseline, 4,203 JHS participants returned for Visit 2. We excluded 427 individuals with prior coronary artery disease, 1099 who were missing obesity and/or inflammation measures from Visit 1 and 580 participants without CAC assessed at Visit 2, resulting in a final analytic sample of 2,097 for the analysis (Supplementary Figure II). Mean CAC and 95% confidence limits were calculated across the inflammatory-adiposity phenotypes and one-way ANOVAs were performed using log-transformed CAC values to compare differences across the four phenotypes. Given the non-normal distribution of CAC scores, they were examined as a multi-categorical variable (calcium score of 0, >0-10, >10-100, >100-300, >300) and as a binary measure (calcium score >0). To model multi-categorical CAC, we used cumulative logistic regression models to estimate the odds of elevated CAC across the four phenotypes using Group 1 as referent. To model binary CAC, we used modified Poisson regression models with a Poisson distribution and a log-link function with robust variance estimator to estimate the prevalence odds ratio (POR) of elevated CAC across the four phenotypes using Group 1 as referent. Regression models were adjusted for age, sex, smoking history, alcohol use, hypertension, hypercholesterolemia, stroke, peripheral vascular disease and diabetes similar to cIMT analyses. Given the significant interaction for gender on the primary outcome, gender-specific analyses were also conducted for CAC even when formal interaction for gender yielded a non-significant result for interaction (p=0.08). Similarly, sensitivity analyses were also conducted using sex- and obesity-specific thresholds for hsCRP.

Statistical significance was set at a 2-sided p<0.05, with the exception of post-hoc within-obesity group comparisons (Group 3 vs. 4) for which the significance was set at p<0.025 to account for multiple comparisons. All analyses were conducted using SAS statistical software (version 9.2).
REFERENCES

In a large African American population-based cohort without known cerebrovascular disease, hsCRP ≥ 2 mg/L did not discriminate the burden of either carotid or coronary atherosclerosis in women. In contrast, hsCRP ≥ 2 mg/L identified a subset of non-obese men with higher burden of carotid atherosclerosis. In presence of obesity, hsCRP < 2 mg/L identified a subset of men with lower burden of coronary atherosclerosis. However, benign obesity did not exist since all phenotypes of obesity were associated with higher carotid atherosclerotic burden in both men and women irrespective of inflammation.