Introduction

Left ventricular (LV) hypertrophy, a thickening of the myocardium of the LV, is one of the most potent risk factors for cardiovascular disease (CVD), including ischemic heart disease, chronic heart failure, and cardiovascular death [1-3]. The thickening of the myocardium in LV hypertrophy is partly due to acute and chronic stress, such as that associated with aortic stenosis and hypertension [3, 4]. Thickness of the LV is correlated with its mass. LV mass can be measured by echocardiography or magnetic resonance imaging (MRI).

Various biological pathways are activated comitantly with the development of LV hypertrophy. For example, several studies have demonstrated associations between inflammatory markers [5] and LV mass [6-9]. Other studies have revealed correlations between hemostatic markers and echocardiographic measures of LV
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structure and function [10, 11]. Although the mechanistic roles of some of these biomolecules have been described [12, 13], their exact causal roles in LV hypertrophy or remodeling are not fully understood. An improved understanding of the associations of these biomarkers with LV structure may offer epidemiologists and clinicians additional tools for risk stratification, provide insight into pathways involved in development of CVD phenotypes, and focus attention on potential therapeutic targets.

The Multi-Ethnic Study of Atherosclerosis (MESA) is one of the largest studies of a racially and ethnically diverse, asymptomatic population wherein inflammatory and hemostatic biomarker data and MRI data, including LV mass, have been collected. In these analyses, we studied the cross-sectional relationships between 13 inflammation and hemostasis biomarkers and LV mass.

Materials and methods

Setting

The MESA was initiated to explore the pathogenesis of atherosclerosis in 4 racial/ethnic groups by accurately assessing early CVD and its progression. Details of the design have been published [14]. Briefly, beginning in July 2000, 6814 men and women age 45-84 years whose self-reported race/ethnicity was white, black, Chinese, or Hispanic were recruited from six US communities: Baltimore City and Baltimore County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; Northern Manhattan and the Bronx, NY; and St. Paul, MN. Participants were included only if they were free of clinically defined CVD, ie, no history of myocardial infarction, angina, coronary revascularization, congestive heart failure, atrial fibrillation, stroke, transient ischemic attack, or peripheral vascular disease. Participation rate among individuals who were screened and eligible was 59.4%. Informed consent was obtained prior to participation; this study complies with the Declaration of Helsinki and institutional review boards of participating centers approved the study.

Baseline clinic visit

Participants underwent an extensive baseline evaluation using standardized questionnaires to obtain information on demographics, CV factors, medical history, and medication use. Height, weight, and waist circumference were measured with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight divided by height² (kg/m²). Resting blood pressure was measured three times in the seated position using a Dinamap model Pro 100 sphygmomanometer (Critikon, Wipro GE Healthcare, Waukesha, WI).

Magnetic resonance imaging

MRI exams were performed on consenting participants; details of the MRI measurements have been described [15, 16]. Briefly, imaging was performed with electrocardiographic gating using a four-element, phased-array surface coil positioned anteriorly and posteriorly and scanners with 1.5-T magnets. Cine images of the LV were read with time resolution <50 msec with the MASS software (version 4.2, Medis, The Netherlands) at a single reading center. Reading center technicians were trained according to the MESA protocol, and readings were conducted in a blinded fashion with respect to the other MESA risk factor or subclinical disease data. MRI variables used to estimate LV mass were restricted to end-diastolic measurements. Associations between ejection fraction (calculated as stroke volume divided by end-diastolic volume) and aortic diameter (measured at the level of the right pulmonary artery) and 13 inflammation and hemostasis biomarkers were also assessed in this study; these associations were generally non-significant and, therefore, are not presented here. For LV mass, technical error of measurement was 6.17% (95% confidence interval [CI] 5.29, 7.04).

Biomarker measurement

Blood biomarkers selected for this study were chosen based on previously reported linkage to cardiovascular disease [7, 17-21].

Participants fasted and avoided heavy exercise for 12 hours prior to venipuncture and were asked to avoid smoking the morning of their exam. All blood samples were processed according to a standard protocol and stored at -80 degree C until analyzed. All the laboratory assays were done at the University of Vermont (Burlington, VT). Table 1 cites the method or
lists the commercial assay used for determination of each biomarker and gives interassay coefficients of variation. Detailed methods have been described elsewhere [23, 24]. CRP, D-dimer, factor VIII, fibrinogen, and IL6 were measured for all MESA participants. Soluble E-selectin, MMP3, MMP9, PAI-1, sTM, sTNFR1, and vWF were measured in a sample of 1000 individuals randomly chosen from the first 5030 MESA participants. sICAM-1 was measured in the first one-third of MESA participants and a 1000-person random sample (n=2600).

Statistical analyses

All analyses were conducted with Stata 11.0 (StataCorp, College Station, TX). The nominal level of significance was defined as P ≤ 0.05 (two-sided tests). Association analyses were performed for the combined race group and for each race stratum. Only interaction analyses significance levels were adjusted for multiple testing. Levels of several biomarkers (CRP, D-dimer, sE-selectin, IL6, MMP3, MMP9, PAI-1, sTNFR1) were heavily skewed and therefore log-transformed to achieve normality prior to analysis. Three linear regression models were used to associate biomarkers with LV mass: model 1 adjusted for age, gender, race/ethnicity, and height. Model 2 adjusted for Model 1 factors plus systolic blood pressure, use of antihypertensive medications, treated diabetes (use of insulin or hypoglycemic agents), current smoking, LDL-C, and statin use. Because of the strong association between inflammation, obesity and LV mass, Model 3 adjusted for model 2 factors plus weight. Height and weight, known to have non-linear relationships with LV mass and some biomarkers, were not log-transformed because models using transformed versus non-transformed body size covariates did not substantially differ. Linearity of associations was examined using scatter plots with superimposed Lowess smoothed curves. Interactions between each biomarker and age, gender, and race/ethnicity on LV mass were also tested using multiplicative terms. Because of the number of analyses (ie, 13 biomarkers, 3 interaction models, total, 39 tests), none of these interactions met a strict Bonferroni-adjusted level of significance (ie, P=0.05/39=0.001), and are not discussed further.

Results

Cardiac MRI capturing technically acceptable images was accomplished for 73% of participants (n=5004). Most missing MRI data was attributed to ineligibility (metallic fragment, implant, or device [7% of 6814]) or inability to

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Assay/Method</th>
<th>CV*(%)</th>
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<tbody>
<tr>
<td>CRP</td>
<td>N High-Sensitivity CRP†</td>
<td>2.1-5.7</td>
</tr>
<tr>
<td>D-dimer</td>
<td>Liatest D-DI‡</td>
<td>8.0</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>Parameter Human sE-Selectin Immunoassay§</td>
<td>5.7-8.8</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>STA-Deficient VIII‡</td>
<td>10.0</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>N Antiserum to Human Fibrinogen†</td>
<td>2.6</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>Parameter Human sICAM-1/CD54 Immunoassay§</td>
<td>5.0</td>
</tr>
<tr>
<td>IL6</td>
<td>Quantikine HS Human IL-6 Immunoassay§</td>
<td>6.3</td>
</tr>
<tr>
<td>MMP3</td>
<td>Quantikine Human MMP-3 (total) Immunoassay§</td>
<td>7.0-8.6</td>
</tr>
<tr>
<td>MMP9</td>
<td>Quantikine Human MMP-9 (total) Immunoassay§</td>
<td>6.9-7.9</td>
</tr>
<tr>
<td>PAI-1</td>
<td>2-site ELISA, DeClerck et al. [22]</td>
<td>3.5</td>
</tr>
<tr>
<td>sTM</td>
<td>Asserachrom Thrombomodulin‡</td>
<td>12.0</td>
</tr>
<tr>
<td>sTNFR1</td>
<td>Quantikine Human sTNF RI Immunoassay§</td>
<td>5.0</td>
</tr>
<tr>
<td>vWF</td>
<td>Liatest vWF‡</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*CVs were calculated in the MESA lab at the time assays were done. Single values refer to instances where one control concentration was analyzed; ranges indicate the range of CVs when multiple control concentrations were analyzed; †Dade Behring, Deerfield, IL; ‡Diagnostica Stago, Parsippany, NJ; §R&D Systems, Minneapolis, MN; CRP, C-reactive protein; D-dimer, fibrin fragment D; sICAM-1, soluble intercellular adhesion molecule-1; IL6, interleukin-6; MMP3, matrix metalloproteinase 3; MMP9, matrix metalloproteinase 9; PAI-1, plasminogen activator inhibitor 1; sTM, soluble thrombomodulin; sTNFR1, soluble tumor necrosis factor, type 1 receptor; vWF, von Willebrand factor.
complete the procedure, usually because of claustrophobia (14%) [25]. Approximately 78% (n=785) of the 1000-person random subsample had technically adequate data cardiac MRI data. Biomarker data were available for approximately 99% of participants.

Table 2 describes baseline characteristics of the full and the random subsample of participants and the individual race groups. The characteristics of participants in the random subsample were similar to those of the full sample in terms of LV mass and the most important correlates (eg, BMI, diastolic BP). All the variables except gender were significantly different among race groups.

The means of the biomarkers, stratified by gender and race, are provided in Table 3. The levels of most biomarkers were significantly different (P ≤ 0.05) across ethnic groups in both sexes. Only MMP3 and sTM (in women) and MMP3, sTM, and vWF (in men) were similar across the races.

Figure 1 shows regression coefficients for linear regressions of each biomarker with LV mass for each of three adjustment models which incorporate traditional LV mass risk factors. Figure 1 also shows incremental increase in R² for the addition of the biomarker to each of the three models and R² for model 3 as well as overall R² for model 3. With the exception of the MMPs, LV
mass was associated with all of the biomarkers listed in Figure 1 either before (models 1 and 2) or after (model 3) adjustment for weight. In general, LV mass coefficients were attenuated (or became non-significant, \( P > 0.05 \)) with increasing covariate adjustment, and \( R^2 \) uniformly decreased with addition of covariates, particularly with addition of weight (model 3). However, the association of LV mass with both CRP and LV mass and IL6 not only changed direction (ie, became negative) after adjustment for weight (model 3), but the negative association was highly significant \( (P < 0.001) \), although \( R^2 \) values were small for both CRP and IL6. See Figure 2 for an illustration of the relationship between CRP and LV mass before and after weight adjustment. A similar pattern was observed for PAI-1, although the inverse association in the weight-adjusted model was not statistically significant. The TNFR1, sICAM-1, D-dimer, and fibrinogen associations with LV mass were all significant \( (P < 0.05) \) after adjustment for risk factors and treatment variables (model 2). Further adjustment for weight (model 3) markedly attenuated these associations and rendered them non-significant. sTM was significantly as-
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associated with LV mass before and after adjustment. Factor VIII and vWF were negatively associated with LV mass, and the associations became stronger and more significant with further risk factor (model 2) and weight adjustment (model 3).

Trends among the models in race/ethnicity-specific analyses were consistent with each other and the non-stratified analysis, including the positive associations of LV mass with CRP and IL6 in models 1 and 2 and negative associations after addition of weight to the model. These data are not shown here.

Figure 1. Beta coefficients (1 unit = 1 standard deviation; error bars show 95% confidence interval (CI)) for regressions between biomarkers and left ventricular (LV) mass (g). From top to bottom in each panel, coefficients for models 1, 2, and 3 are indicated. (See text for explanation of adjustment models) The number in the lower right corner of each panel represents 1 standard deviation in the units indicated under the biomarker name. $\Delta R^2$ column shows the incremental change in R-squared when adding the biomarker to models 1, 2, and 3, respectively. $R^2$ column shows model 3 R-squared, with corresponding biomarker included. CRP, c-reactive protein; D-dimer, fibrin fragment D; IL6, interleukin-6; PAI-1, MMP3, matrix metalloproteinases 3; MMP9, matrix metalloproteinase 9; plasminogen activator inhibitor 1; sE-selectin, soluble E-selectin; sTM, soluble thrombomodulin; sTNFR1, soluble tumor necrosis factor type 1 receptor; siCM-1, soluble intercellular adhesion molecule-1; vWF, von Willebrand factor.

Figure 2. Illustration of the relationship between CRP and LV mass before and after weight adjustment. Plots are based on smoothed values and 95% confidence intervals from generalized additive models with and without weight. Model 2 covariates were used with all continuous variables modeled using 3 degree of freedom smooth terms including age, systolic blood pressure, height, log CRP, and (for lower panel) weight.
Discussion

We hypothesized that increased LV mass is associated with increased inflammation and a hypercoagulable state, and confirmed that, in this large multiethnic cohort, LV mass was associated with several inflammation and hemostatic biomarkers even after adjustment for demographic characteristics, traditional cardiovascular risk factors, treatment for diabetes, hypertension, statin use, and body weight. The associations were attenuated and became non-significant for sTNFR1, sICAM-1, sE-selectin, D-dimer, fibrinogen, and PAI-1 after adjustment for all factors, including weight. Factor VIII and vWF were negatively associated with LV mass in all models, and these same adjustments increased the strength of the negative association between LV mass and factor VIII and vWF. sTM was modestly associated with LV mass, and the relationship was somewhat attenuated with increasing levels of adjustment. Surprisingly, incremental adjustment of our regression models (ie, the addition of weight to the models) changed the direction of regression coefficients for CRP and IL6 (ie, from positive to negative). Given the cross-sectional design of this analysis, we can only speculate regarding why this occurred. One explanation is that the association between these biomarkers and LV mass is mediated through adiposity (ie, weight is associated with both inflammation and LV mass, and adjustment for weight materially changed the association between inflammation and LV mass). Another possible explanation is that in clinically healthy people where both LV mass and CRP are normal (the majority of the MESA participants), where LV hypertrophy is just beginning but still is in the preclinical state, there is a compensatory decrease in inflammatory markers. In this scenario, as LV hypertrophy develops and becomes clinically manifest, systemic inflammation manifests, which would increase inflammatory and hemostatic markers. These analyses are intentionally biased towards this early phase (ie, an inverse relation between CRP and IL6 and LV mass). If either of these explanations is true, then the apparent inverse association of CRP and IL6 with LV mass after adjustment for weight is a novel finding and warrants further examination.

The acute-phase protein CRP has been studied intensively in the context of LV mass and geometry; however, study samples and designs have varied widely, and findings have been inconsistent [26, 27]. In aggregate, these studies suggest that association between CRP and LV mass is likely indirect, perhaps reflecting the association of body size and LV mass. As noted above, our findings, specifically that higher CRP is statistically associated with lower LV mass after controlling for weight, suggests a more complicated association of this acute phase reactant on LV mass in healthy individuals free of clinically apparent CVD.

Since IL6, a pro-inflammatory cytokine secreted by T cells and macrophages, is the major inducer of CRP gene expression and promotes a rise in blood levels of the protein product, an association between IL6 and LV mass similar to that of LV mass and CRP is not unexpected. Indeed, we observed consistent results between CRP and IL6 and LV mass, ie, a positive association between these two biomarkers and LV mass after adjustment for demographic and risk factors, and a change of the association from positive to negative after adjustment for adiposity. This comparable behavior of IL6 and CRP in our models suggests that the association is not a spurious one, but rather indicates a more complex relationship between these inflammatory markers, adiposity and LV mass. Like CRP, plasma concentrations of IL6 correlate with body mass index and percent body fat [28]. Circulating plasma levels of both IL6 and CRP might reflect local synthesis of these molecules by adipocytes in addition to production in the liver. It is plausible that the inverse association between LV mass and IL6 and CRP reflects a physiological response of the LV to both local (eg, pericardial fat) and systemic (hepatic) CRP production. Observational studies have reported significant positive correlations between IL6 concentrations and echocardiographically determined LV end-systolic volume and LV ejection fraction in clinically identified samples with LV dysfunction or heart failure [29]. In a study of patients who underwent cardiac catheterization, Raymond et al. found IL6 concentrations were elevated in patients with LV dysfunction, including those without clinical HF symptoms [30]. Orus et al. reported that IL6 was an independent predictor of death or a new heart failure episode in patients with LV dysfunction [31]. These findings may differ from ours because their study populations had evident CVD, while the MESA participants were free of clinical CVD.

We observed a significant positive associations between sTM, indicating greater endothelial
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dysfunction, and LV mass that were only slightly attenuated by adjustment for risk factors, treatment, and weight. We found no studies that examined associations between sTM and LV mass, and given the cross-sectional nature of our analysis, we can only speculate that processes accompanying endothelial damage that relate to increased LV mass might upregulate TM expression on damaged endothelium.

We observed inverse associations for both vWF and Factor VIII with LV mass which increased in magnitude and level of significance with adjustment for risk factors and their treatment, as well as weight. This is in contrast to other reports from smaller samples of diseased subjects [32, 33].

Five hemostatic/inflammatory biomarkers (ie, sTNFR1, sICAM-1, D-dimer, fibrinogen, and PAI-1) were associated with LV mass, although all were attenuated by adjustment for adiposity (model 3) but not CVD risk factors or treatment (model 2). All of these biomarkers are correlated with adiposity and are determinants of whole blood viscosity or inflammation, which are related to both adiposity and LV mass [34, 35]. Therefore, it was not unexpected that adjustment for weight might completely remove the observed association of these five biomarkers with LV mass. While these results suggest that hemostatic and inflammatory parameters likely reside in the causal pathway linking obesity to LV mass, it may be that elevated levels of these markers induce weight gain, as suggested by Duncan et al [36]. Further work to disentangle the effects of these hemostatic variables on LV mass independent of weight is needed.

We did not find associations between LV mass and MMP3 or MMP9. This was unexpected given the number of publications that have reported positive associations between MMPs and LV mass [37, 38]. As previously suggested for CRP and IL6, it is plausible that the MESA population is healthier and younger than other studies reported previously, and that these markers become positively associated later in disease progression. The sample size was smaller for this subset of biomarkers, and the study could have been inadequately powered to detect very modest associations.

This study has a number of limitations. As a cross-sectional description of relationships between biomarkers and LV mass, the temporal association and causal relationship between these biomarkers and LV mass is unclear. It is possible that both reflect a shared process, such as obesity and/or atherosclerosis, which results in their co-occurrence. Additionally, our sample size for several biomarkers (~700 subjects) was smaller than our overall sample; nonetheless, it is one of the largest collections of these markers and MRI measures available to date. We previously reported strong associations between traditional risk factors, such as higher blood pressure and body mass index and MRI LV mass in MESA participants; these risk factors may be particularly important in the early stages of the development of LV hypertrophy [25]. These traditional risk factors explain a much larger proportion of variation in LV mass than these biomarkers where the change in R² values observed with the inclusion of the biomarker ranged from 0.001 to 0.01 in the least adjusted models.

Strengths of the study include the measures of LV mass with MRI, a highly accurate and reproducible method for measurement. MESA represents a large, multi-ethnic population free of clinically-apparent CVD, and we found that associations were similar in all four ethnic groups.

In this cohort free of clinical cardiovascular disease, hemostasis and inflammation markers were associated with LV mass. Adjustment for demographic variables and risk factors attenuated many of these associations. A surprising finding was that incremental additional adjustment of our regression model changed the direction of beta coefficients for LV mass with CRP and IL6 (ie, from positive to negative). Future analyses to evaluate the longitudinal relationship of these biomarkers and LV mass as well as other measures of cardiac structure and function are necessary.

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Institutes of Health.

Conflict of interest statement

Dr. Szalai has received funding from Isis Pharmaceuticals, Inc. of Carlsbad, California for research concerning a drug intended to lower clinical concentrations of one of the biomarkers described here. All other authors declare no potential conflicts of interest.

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