Mediation of Cardiovascular Risk Factor Effects Through Subclinical Vascular Disease
The Multi-Ethnic Study of Atherosclerosis


Objective—It is unclear to what extent subclinical cardiovascular disease (CVD) such as coronary artery calcium (CAC), carotid intima-media thickness (CIMT), and brachial flow-mediated dilation (FMD) are mediators of the known associations between traditional cardiovascular risk factors and incident CVD events. We assessed the portion of the effects of risk factors on incident CVD events that are mediated through CAC, CIMT, and FMD.

Approach and Results—Six thousand three hundred fifty-five of 6814 Multi-Ethnic Study of Atherosclerosis participants were included. Nonlinear implementation of structural equation modeling (STATA mediation package) was used to assess whether CAC, CIMT, or FMD are mediators of the association between traditional risk factors and incident CVD event. Mean age was 62 years, with 47% men, 12% diabetics, and 13% current smokers. After a mean follow-up of 7.5 years, there were 539 CVD adjudicated events. CAC showed the highest mediation while FMD showed the least. Age had the highest percent of total effect mediated via CAC for CVD outcomes, whereas current cigarette smoking had the least percent of total effect mediated via CAC (percent [95% confidence interval]: 80.2 [58.8–126.7] versus 10.6 [6.1–38.5], respectively). Body mass index showed the highest percent of total effect mediated via CIMT (17.7 [11.6–38.9]); only a negligible amount of the association between traditional risk factors and CVD was mediated via FMD.

Conclusions—Many of the risk factors for incident CVD (other than age, sex, and body mass index) showed a modest level of mediation via CAC, CIMT, and FMD, suggesting that current subclinical CVD markers may not be optimal intermediaries for gauging upstream risk factor modification. (Arterioscler Thromb Vasc Biol. 2014;34:1778-1783.)

Key Words: cardiovascular events ■ carotid intima-media thickness ■ coronary artery calcium score ■ flow-mediated dilation ■ risk factors

It is widely assumed in cardiovascular medicine that the effects of traditional risk factors on clinical cardiovascular events are largely a consequence of their effects on anatomic or functional vascular disease.1 In this view, much of the risk from conventional risk factors is mediated through subclinical disease. This line of reasoning, which is amply supported by animal and human experimental data,2–4 prompted several decades of intermediate disease end point clinical trials5,6 with the expectation that changes in subclinical vascular disease would foreshadow the ultimate clinical benefit of specific risk factor interventions. It is notable, however, that the actual use of this approach has been highly variable.3 Recently, this emphasis on subclinical vascular disease has been expanded to include the incremental value of subclinical disease markers above and beyond traditional risk factors for risk prediction although, in general, the incremental contributions have been modest.5,6 The clinical corollary to this conceptual model is that measures of subclinical disease could be used to help gauge how aggressive to be in the setting of borderline abnormal risk factors or when there are competing clinical factors that might mitigate against more aggressive therapy (eg, statin intolerance). Patients with risk factors but no subclinical disease could be considered relatively resistant to the effects of the risk factors themselves and therefore may require less aggressive intervention.

Despite the intuitive appeal of this paradigm, there are, in fact, few data that systematically quantify the contribution of the effects of risk factors mediated through anatomic or functional measures of vascular disease versus effects that may operate via other mechanisms such as inflammation, plaque ulceration, thrombus formation, or other novel pathways.10 However, recent improvements in structural equation

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modeling provide a statistical framework to effectively partition mediated effects (ie, effects operating through a measurable intermediary) from effects that are either directly attributable to the risk factor or to other unmeasured mediating pathways. Such partitioning of risk attributable to anatomic or functional measures of vascular disease (mediated effects) versus effects that are independent of these measures may provide clues to additional mechanisms of action of conventional risk factors and emphasize areas where more precise or physiologically specific measures of subclinical disease are needed. Clinically, estimating risk from risk factors that is mediated through, or modified by, measures of subclinical disease could help clinicians determine how much weight to place on these measures when making treatment recommendations for primary prevention.

Accordingly, we used structural equation models and conventional interaction analyses to analyze the relationship between conventional cardiovascular disease (CVD) risk factors, measures of subclinical disease (including coronary artery calcium [CAC] score, carotid intima-media thickness [CIMT], and brachial flow-mediated dilation [FMD]), and risk for clinical cardiovascular events in the Multi-Ethnic Study of Atherosclerosis.

Materials and Methods
Materials and Methods are available in the online-only supplement.

Results
A total of 6355 participants (3336 for brachial FMD) had complete data on the covariates of interest and were included in this analysis. The mean age and body mass index were 62 years and 28 kg/m², 47% were men, 39% whites, 12% Chinese, 26% black, 23% Hispanics, and 12% had diabetes mellitus. Table 1 shows the demographic and risk factors stratified by sex. After a mean follow-up of 7.5 years (maximum of 9 years), there were 539 CVD and 388 coronary heart disease (CHD) adjudicated events.

Are CAC, CIMT, and FMD Mediators of the Association Between Traditional Risk Factors and Incident CVD and CHD?
Table 2 shows the overall effect of traditional cardiovascular risk factors on incident CVD events as well as the portion of those effects mediated through the subclinical disease measures (CAC, CIMT, and brachial FMD). For example, for CVD events (Table 2, Row 4), a 1-SD (10-year) increase in age was associated with an increase of 33.6 events/10000 person-years. Of that overall effect, 14.6 events/10000 person-years (43.3%) was mediated through the presence of CAC. The remainder (19.0 events/10000 person-years) was either a direct effect of age or an effect mediated through other unmeasured intermediary pathways. All of the other risk factors had a significant but considerably smaller portion of their effects mediated through the presence of CAC (range, 5.9–27.1%). Figure 1 is a diagram illustrating the portions of the effect of a 1-SD increase in body mass index on clinical CVD events that is mediated via the presence of CAC versus that mediated via other pathways.

The continuous measure of coronary calcium [log (CAC+25)] accounted for a larger portion of the risk associated with the risk factors (except high-density lipoprotein cholesterol), including 80.2% of the risk associated with 1-SD (10-year) increase in age. However, even with this more precise measure of vascular disease, no more than 52.2% of the risk associated with any of the other risk factors or the Framingham Risk Score (FRS) was mediated through this subclinical disease measure.

In the case of CIMT, the traditional risk factor with the highest mediation was body mass index (17.7%). The portion of the total effect of the FRS on incident CVD mediated via CIMT was only 7.4%. Only 2.0% of the effect of a 1-SD (10-year) increase in age on risk for CVD was mediated through brachial FMD. For all the other risk factors, the effects...
mediated through brachial FMD were negligible. Similar patterns of total effects/10,000 person-years and percent of total effect mediated via CAC, log (CAC+25), CIMT, and FMD were observed for CHD events (Table I in the online-only Data Supplement).

Do CAC, CIMT, and FMD Modify the Association Between Traditional Risk Factors and Incident CVD or CHD?

Table 2 shows the extent to which the effects of traditional risk factors were modified by measures of subclinical vascular
disease (CAC, CIMT, and FMD). Overall, the evidence for risk factor effect modification was almost exclusively limited to the continuous measure of coronary calcium, and the direction of the interaction effect was counter to the prevailing subclinical disease paradigm. Thus, for example, the risk associated with a 10% increase in the FRS score was greater when the coronary calcium score was low than when the coronary calcium score was high (Figure 2). Similarly, the risk associated with a 1-SD increase in age or systolic blood pressure was greater when coronary calcium score was low than when the coronary calcium was high. The risk associated with male sex was less when CIMT was less than when CIMT was high. In no instance was there evidence that risk associated with any risk factor was attenuated by having less coronary calcium, carotid wall thickening, or impaired FMD. There was no statistically significant evidence for interaction between the risk factors and subclinical disease measures on risk for CHD events (Table II in the online-only Data Supplement).

**Discussion**

The goal of this study was to determine the degree to which measures of subclinical CVD such as CAC, CIMT, and FMD mediate or moderate the known association between traditional cardiovascular risk factors and incident CHD or CVD events. We found that among the risk factors studied, age was the one whose relationship with risk for events was most clearly mediated through subclinical disease measures. Indeed, >80% of the risk associated with age could be accounted for by the coronary calcium score \[\log (CAC+25)\]. Age was also the risk factor best accounted for by the other subclinical disease measures although considerably less of the age-associated risk could be explained by CIMT and only a trivially small amount of the age-associated risk was mediated through FMD. To our knowledge, this is the first study that has evaluated the portion of the effects of current traditional risk factors on clinical cardiovascular events that are mediated via FMD. To our knowledge, this is the first study that evaluated the portion of the effects of current traditional risk factors on clinical cardiovascular events that are mediated via FMD. To our knowledge, this is the first study that evaluated the portion of the effects of current traditional risk factors on clinical cardiovascular events that are mediated via FMD. To our knowledge, this is the first study that evaluated the portion of the effects of current traditional risk factors on clinical cardiovascular events that are mediated via FMD.

In dramatic contrast, for the other risk factors considered the subclinical disease measures were a poor proxy for the risk factors themselves. Even when the effects of the risk factors were aggregated in the form of the FRS, the best subclinical disease measure \[\log (CAC+25)\] accounted for only 42% of its effect on risk for CVD events. Furthermore, there was no evidence from the interaction analyses to suggest that the effects of any risk factor could be discounted because of the absence of significant subclinical disease.

Collectively, these data call into question the use of these subclinical disease measures as intermediate or surrogate markers of disease risk attributed to the risk factors themselves. Although not studied here, these data naturally raise questions about the extent to which change in these subclinical disease measures would be expected to forecast the clinical effects of change in the upstream risk factors.12–14 Questions about the usefulness of subclinical disease measures as a proxy for the effects of risk factors are not new; however, the data here provide for the first time formal estimates of the actual portions of risk mediated by subclinical disease measures and suggest that the degree of mediation may be less, in some cases considerably less, than expected.

Importantly, these data do not suggest that the subclinical disease measures are uninformative. Indeed, there was evidence that both coronary calcium score and CIMT mediated at least some risk for every risk factor studied. However, except for age, sex, and body mass index, the mediated effects were small—the bulk of the effects of the conventional cardiovascular risk factors seem to operate through pathways or mechanisms that are not adequately reflected by coronary calcium, CIMT, or FMD. Numerous studies from this and other cohorts have documented the modest but measurable incremental value of subclinical disease measures for prediction of clinical events above and beyond conventional risk factors.15,16 Presumably, these incremental effects are mediating effects of other known or unknown risk factors. However, the degree to which these subclinical disease measures are an adequate reflection of the risk associated with these other known and unknown risk factors remains to be determined.

The inferences here relate to statistical mediation and modification by measures of anatomic or functional vascular disease. The quality of the evidence that anatomic or functional abnormalities are in the causal pathway between risk factors and clinical events is extremely strong.17 However, it is possible that current subclinical disease measures are focused on the wrong anatomic or functional features or produce measures that are too imprecise to be of much value as proxies for risk factors themselves. FMD, for instance, is well known to have significant measurement error,18 even though the biological validity of endothelial function as an antecedent to atherosclerosis and clinical events is high. Unfortunately, even coronary calcium, which is a considerably more precise measure, does not seem to capture a substantial portion of the risk attributable to several important risk factors including smoking, dyslipidemia, and hypertension. Newer imaging approaches designed to identify lipid-laden or vulnerable plaques may prove to be more useful than the subclinical disease measures studied here.19 However, more work is needed to document their use for risk prediction and to make them more widely available to the practicing clinical community. Many risk factors likely also have effects on inflammation or propensity for thrombosis—effects that would not be expected to be captured by measures of anatomic manifestations of atherosclerosis.

The strengths of our study include the large sample size, the multiethnic composition of the cohort, the standardized acquisition and centralized readings of subclinical disease measures, the high-quality event surveillance and adjudication, and the novelty of the analytic approach. Some of the mediated and nonmediated effects of plasma lipids and blood pressure were likely influenced by use of antihypertensive and lipid lowering therapies, which could not be easily accounted for in our mediation models. In addition, our models were based on measures...
of risk factors and subclinical disease measures from a single point in time and do not reflect risk associated with lifetime exposures to risk factors or changes in subclinical disease over time. Measurement errors in both risk factors and subclinical disease likely attenuated the certainty of the estimates of risk, mediation, and modification. Despite this, most of the estimates of mediation for coronary calcium and intima-media thickness were statistically significant, although frequently rather small.

**Conclusions**

With the exception of age and sex, the majority of the effects of traditional cardiovascular risk factors on risk for clinical cardiovascular disease events were mediated by subclinical disease measures.
events are not reflected in measures of coronary calcium, carotid thickness, and brachial FMD. Furthermore, there is no evidence that these subclinical disease measures could be used to discount the anticipated risk associated with the conventional risk factors. These data re-emphasize the central importance of conventional risk factors for risk prediction and highlight the need for improved means to quantify the intermediate steps in the pathways from risk factors to clinical events.

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Disclosures

None.

References


Significance

Despite their extensive use as intermediate end points in clinical cardiovascular disease (CVD) trials, it is unclear to what extent subclinical CVD markers such as coronary artery calcium, carotid intima-media thickness, and brachial flow-mediated dilation are mediators of the known associations between traditional cardiovascular risk factors and incident CVD events. We used coronary artery calcium, carotid intima-media thickness, flow-mediated dilation, traditional CVD risk factors, and 7.5 years of adjudicated CVD events in participants who were part of the Multi-Ethnic Study of Atherosclerosis to show that modest effects of CVD risk factors (especially the modifiable CVD risk factors) on incident CVD events are mediated via these subclinical CVD markers. This suggests that current subclinical CVD markers (coronary artery calcium, carotid intima-media thickness, and flow-mediated dilation) may not be optimal intermediaries for gauging upstream risk factor modification in clinical trials.
Mediation of Cardiovascular Risk Factor Effects Through Subclinical Vascular Disease: The Multi-Ethnic Study of Atherosclerosis

Joseph Yeboah, Joseph A. Delaney, Robin Nance, Robyn L. McClelland, Joseph F. Polak, Christopher T. Sibley, Alain Bertoni, Gregory L. Burke, J. Jeffery Carr and David M. Herrington

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Methods

Study Population and Data Collection

A detailed description of the study design for MESA has been published elsewhere (1). In brief, MESA is a prospective cohort study begun in July 2000 to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease (CVD) in individuals without known CVD at baseline. The cohort includes 6814 women and men aged 45–84 years old recruited from 6 US communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and St. Paul, MN). MESA participants were 38% white (n = 2624), 28% black (n = 1895), 22% Hispanic (n = 1492) and 12% Chinese (n = 803). Individuals with a history of physician-diagnosed myocardial infarction, angina, heart failure, stroke or transient ischemic attack, or who had undergone an invasive procedure for CVD (coronary artery bypass graft, angioplasty, valve replacement, pacemaker placement or other vascular surgeries) were excluded. Demographics, medical history, anthropometric and laboratory data for this study were obtained at the first MESA examination (July 2000 to August 2002). Self-reported race/ethnicity was collected to explore the possible racial differences in the development and progression of atherosclerosis. Current smoking was defined as having smoked a cigarette in the last 30 days. Diabetes mellitus was defined as fasting glucose ≥126 mg 100 ml$^{-1}$ or the use of hypoglycemic medications. Use of antihypertensive and other medications was based on the review of prescribed medication containers. Resting blood pressure was measured using an automated oscillometric blood pressure device (Dinamap PRO 100®) three times in seated position, and the average of the second and third readings was recorded. Body mass index was calculated as weight (kg)/height$^2$
(m²). Total and high-density lipoprotein cholesterol were measured from blood samples obtained after a 12-h fast. Low-density lipoprotein cholesterol was estimated by the Friedewald equation (2). The MESA study was approved by the institutional review boards of each study site, and written informed consent was obtained from all participants.

**Measurement of Coronary Artery Calcium (CAC) Score**

Details of the MESA computerized tomography (CT) scanning and interpretation methods have been reported by Carr et al (3). Scanning centers assessed CAC by chest CT with either a cardiac-gated electron-beam CT scanner (Chicago, Illinois; Los Angeles, California; and New York, New York field centers) or a multi-detector CT system (Baltimore, Maryland; Forsyth County, North Carolina; and St Paul, Minnesota field centers). Certified technologists scanned all participants twice over phantoms of known physical calcium concentration. A radiologist or cardiologist read all CT scans at a central reading center (Los Angeles Biomedical Research Institute at Harbor–UCLA, Torrance, California). We used the mean Agatston score for the 2 scans in all analyses (4) Intra-observer and inter-observer agreements were excellent (κ = 0.93 and κ = 0.90, respectively).

**Measurement of Carotid Intima-Media Thickness**

The details for carotid intima-media thickness (CIMT) measurement and interpretation have been reported by Polak et al (5). The mean of maximum intima-media thickness of the common carotid artery was used. Reproducibility was assessed by blinded replicate readings of CIMT performed by 2 readers. One reader re-read 66 studies, for a between-reader correlation coefficient of 0.84 (n=66), and the other re-read 48 studies, for a correlation coefficient of 0.86. The
re -scan and the re-read coefficient of variation were 7.07% and 3.48% respectively.

**Measurement of Brachial Flow Mediated Dilation**

Methods for the MESA brachial flow mediation dilation (FMD) measurement and interpretation have been reported by Yeboah et al (6). Intrareader reproducibility for baseline diameter, maximum diameter, and %FMD was evaluated by comparing an original and a blinded quality control reread of ultrasounds from 40 MESA participants. The intraclass correlation coefficients (ICC) were 0.99, 0.99, and 0.93, respectively. Intrasubject variability was evaluated by comparing results from repeated examinations of 19 subjects on 2 days a week apart. The ICC for baseline diameter, maximum diameter, and %FMD were 0.90, 0.90, and 0.54, respectively. Percent technical error of measurement was 1.39% for baseline diameter measurement, 1.47% for maximum diameter measurement, and 28.4% for %FMD measurement.

**Ascertainment of Incident CHD and CVD**

For the purpose of this report we included all clinical cardiovascular events that occurred through May 2011. CVD events were adjudicated by a MESA committee that included cardiologists, physician epidemiologists, and neurologists. A detailed description of the adjudication process has been published (6). For the purposes of this study, we define incident coronary heart disease (CHD) as myocardial infarction (MI), CHD death, resuscitated cardiac arrest, definite or probable angina if followed by coronary revascularization. Incident CVD additionally included stroke or CVD death as defined by the MESA protocol (www.mesa.nhlbi.org). Thus CHD is a subset of CVD. CVD and CHD were used as outcomes in our models. We did not include stroke as an outcome due to the
relatively low number of adjudicated strokes in this cohort at the time of this analysis and the fact that complex approaches such as SEM typically require a fair bit of power to overcome convergence issues.

**Statistical Analysis**

For the purpose of this analysis, we considered coronary calcium as both a binary variable (coronary calcium present or absent) and as a log-transformed continuous variable \([\log (\text{CAC} + 25)]\) following Kronmal et al. (7). Both carotid IMT and brachial FMD were treated as non-transformed continuous variables. Models involving brachial FMD were weighted to reflect the non-random sampling of MESA subjects that were included in the brachial FMD ancillary study. The mediation analysis was performed using the non-linear implementation of structural equation modeling of Imai et al. (8) implemented in the mediation package for STATA. This approach allowed us to include interactions in the mediation model (if any important interactions were found) as it relaxes the “no interactions” assumption of traditional mediation analysis. The STATA package also allowed us to test how robust our estimates of mediated and direct effects were to unmeasured confounders and measurement error. Finally, this package permits the use of sampling weights for the FMD analysis. Cox models are not directly implemented in the STATA mediation package so we tested pooled logistic regression models and linear regression models to approximate the time to event piece of the Cox model estimates (9). Testing of different periods of pooling indicated that straightforward logistic regression models and linear regression models were uniformly consistent with the pooled logistic regression models yielding less than 4% difference in estimates in the
most extreme case. We tested each of the potential mediators (CAC, CIMT, and FMD) sequentially for each of the risk factors (age, gender, systolic blood pressure, total and HDL cholesterol levels, current cigarette smoking, BMI and diabetes mellitus) to estimate the portion of the effects of the risk factors that were mediated or not mediated through the subclinical disease measures. For non-binary risk factors (such as age), we used only linear models to develop the final mediation models as it was unclear that continuous treatments would generalize to non-linear outcomes (10). The linear structural equation models for each individual risk factor were conditioned on all of the remaining risk factors. We also used the Framingham risk score (constituents in the equation: sex, age, blood pressure, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), smoking behavior, and diabetes status) (FRS) (11) to evaluate mediation of the aggregate effect of all of the risk factors under consideration. Robust methods were used to estimate all confidence intervals for the linear structural equation models. We also tested for interaction between individual risk factors and subclinical disease measures using multiplicative interaction terms in traditional Cox models after adjustment for age, gender, race/ethnicity, BMI, total and HDL cholesterol, systolic and diastolic blood pressure, triglycerides, cigarette smoking status, statin and BP med use, aspirin use and socio economic status. All continuous measures (age, BMI, total and HDL cholesterol, systolic blood pressure) were standardized (variable ÷ standard deviation) prior to entry into our models and are presented as such in the tables and text.
References:


