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Multi-Ethnic Study of Atherosclerosis: Biomarkers of Key Biological Pathways in Cardiovascular Disease

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Abstract

This review provides background on the laboratory design for the Multi-Ethnic Study of Atherosclerosis (MESA) as well as the approach used in MESA to select biomarkers for measurement. The research related to the multitude of circulating and urinary biomarkers of inflammation and other novel and emerging biological pathways in MESA is summarized by domain, or pathway, represented by the biomarker. The contributions of MESA biomarkers to our knowledge of these key pathways in the development and progression of atherosclerosis, cardiovascular disease (CVD), diabetes, kidney disease and pulmonary disease are highlighted as are the contributions of MESA to recommendations for clinical use of several of these biomarkers. In addition, contributions of MESA to multi-cohort genomics consortia and current collaborations in trans-omics and metabolomics are noted.

Laboratory Design for the Multi-Ethnic Study of Atherosclerosis

At the baseline exam (2000-2002), three groups were designated: Group 1 assays were performed on all participants on-line with results returned to participants; Group 2 assays were performed on all participants at the end of the examination; and Group 3 assays were performed on a selected subset of participants, also at the end of the examination. Group 1

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assays included plasma lipid measurements (total cholesterol, high density lipoprotein (HDL) cholesterol and calculated low density lipoprotein (LDL) cholesterol and triglycerides), serum creatinine and fasting glucose. Group 2 measurements included urinary albumin and creatinine, total homocysteine, inflammation markers (interleukin-6 (IL-6), C-reactive protein (CRP), fibrinogen), fasting insulin, several hemostasis and fibrinolysis markers (factor VIII, D-dimer and others) and individual lipoprotein subclasses by nuclear magnetic resonance (NMR LipoProfile-II spectral analysis).

Group 3 was created to allow for extensive phenotyping which was more experimental in nature and included 1,000 participants randomly selected from the 5,030 MESA participants enrolled prior to February, 2002. Due to differences in recruitment rates, this subgroup was 57% women, 46% White, 10% Chinese-American, 21% African-American and 23% Hispanic (the full MESA cohort was 53% women, 38% White, 12% Chinese-American, 28% African-American and 22% Hispanic). Group 3 assays reflected novel and emerging pathophysiological domains and included biomarkers of inflammation, endothelial function, oxidative damage and stress, atherosclerotic plaque stability and chronic infection serologies. Subsequently, 1,880 participants were added to Group 3 to create a subgroup of 2,880 participants comprised of 720 participants from each ethnic group matched for age and sex.

Additional biomarker measurements were conducted by ancillary studies including measurements of circulating immune cells, adipokines, renin and aldosterone, vitamin D metabolites and related analytes, stress hormones and sex hormones. A comprehensive list of the MESA main and ancillary studies biomarker measurements by specific domain (biological pathway) represented by the biomarker, with the number of measurements available at each examination, is presented in Table 1. Full references for each section are presented in an online supplement.

Domain-Based Approach to Biomarker Selection

MESA was designed to investigate the prevalence and progression of subclinical CVD and to identify risk factors for incident clinical CVD in a racially/ethnically diverse population. Therefore, the initial focus of biomarker selection was biological pathways, or domains, intimately involved in the development and progression of CVD. Domains of inflammation, insulin resistance, lipids, hemostasis/fibrinolysis, oxidative damage and stress, endothelial cell function and several others were selected by committee as key pathways at the beginning of the MESA study. Over time, measurements have expanded to include biomarkers representative of other domains such as adaptive immune function and plaque destabilization. Many of these pathways are important not only in CVD, but in other chronic inflammatory diseases such as diabetes, renal disease and pulmonary disease. These common pathways are of particular interest as they may help define the excess risk of CVD in patients with other inflammatory diseases. As with initial biomarker selection, measurements were vetted by committee with consideration given to potential significance of research findings, impact of sample use on the repository and appropriateness of the MESA cohort for the research.

Biomarker Domains Measured in MESA

Inflammation

Inflammation biomarkers measured represent both general inflammation and several specific inflammatory pathways. CRP is perhaps the best known of these systemic inflammation markers. CRP is a non-specific acute phase protein synthesized by hepatocytes, arterial smooth muscle cells and adipocytes in response to inflammatory cytokines such as interleukin-6 (IL-6). Data from MESA has contributed to the status of CRP as a nontraditional marker for CVD risk with clinical utility in screening. CRP was found to identify asymptomatic individuals at higher risk of a CVD event than predicted by traditional risk screening guidelines. Gender differences in CRP levels were also noted, with women having higher CRP levels than men across all race/ethnic groups, suggesting that clinical cut points should be sex-specific[1]. In addition, CRP was found to be an independent predictor of myocardial functional deterioration in asymptomatic individuals with no history of heart disease.

IL-6 is a pro-inflammatory cytokine with multiple humoral and cellular effects. As a direct regulator of the inflammation response, IL-6 may serve as a link between inflammation and CVD. Studies in MESA support a strong association of IL-6 with left and right ventricular function and endothelial function[2].

Fibrinogen is a major circulating pro-coagulant protein, a non-specific acute phase reactant and also an inflammation biomarker. In MESA, elevated fibrinogen levels were associated with impaired myocardial systolic function supporting the interplay of inflammation, coagulation and hyperviscosity in the pathogenesis of myocardial dysfunction.

Biomarker measurements also included several novel biomarkers such as pentraxin 3 (PTX3). PTX3 is related to CRP, but is produced at sites of inflammation by vascular endothelial cells, smooth muscle cells and macrophages and is thought to be a specific marker of vascular inflammation. In MESA, PTX3 was associated with CVD risk factors, subclinical CVD measures, coronary artery calcium (CAC) and incident coronary heart disease (CHD) independent of CRP. Associations of PTX3 with greater right ventricular mass and larger right ventricular end-diastolic volume suggest a functional role for PTX3 in the pulmonary circulation-right ventricular axis[3]. PTX3 has also been associated with kidney dysfunction and highlights the importance of endovascular inflammation in early kidney dysfunction, particularly in blacks.

Insulin Resistance

Levels of glycosylated hemoglobin A_1C (HgA_1C), which provide information on glycemic status, were associated with measures of subclinical CVD in non-diabetic MESA participants. These results suggest that a clinical definition of diabetes based on fasting glucose levels alone may not represent the true level of cardiovascular risk due to impairments in glucose regulation.

Lipids and Fatty Acids

Lipoprotein (protein-lipid complex) particle subclass concentrations were measured by NMR spectroscopy. Small and medium diameter HDL particles were found to be inversely associated with risk of CHD and noncardiovascular, noncancer chronic inflammation-related death and hospitalization and CHD in MESA, suggesting that smaller HDL particles may have anti-inflammatory properties. Conversely, small LDL particles were associated with increased CHD risk. In addition, LDL particle number was a better estimator of atherosclerotic risk when there was discordance between LDL cholesterol (mass of cholesterol carried by LDL particles) and LDL particle number[4].

Levels of lipoprotein(a) (Lp(a)), a well-characterized subspecies of LDL, are not influenced by lifestyle factors but are instead strongly influenced genetically, leading to noted racial/ethnic differences in circulating Lp(a). Extending these findings, associations of Lp(a) with CHD in MESA suggested that clinical cutoffs for Lp(a) should be race/ethnic-based[5]. In addition, MESA was the lead cohort in a multi-cohort consortium examining genes for aortic calcification which identified variation in the Lp(a) gene locus (LPA) as potentially causative.

Dietary fatty acids have been associated with a number of outcomes in MESA. Pentadecanoic acid, a fatty acid biomarker of dietary dairy intake, was inversely associated with incident CVD and CHD, suggesting a potential cardio-protective role for dietary dairy fat. Similarly, *trans*-palmitoleic acid from dairy fat was associated with lower blood pressure and lower risk of incident diabetes.

Circulating levels of polyunsaturated omega-3 fatty acids derived from dietary seafood were inversely associated with CVD incidence supporting the hypothesis that increased consumption of omega-3 fatty acid-rich seafood may be beneficial in CVD prevention. Omega-3 fatty acids were likewise inversely associated with lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a biomarker of atherosclerotic plaque stability, indicating a potential mechanism for the cardiovascular benefits of these dietary fatty acids.

Hemostasis and Fibrinolysis

Statin use in MESA was associated with lower levels of D-dimer, a fibrin degradation product and biomarker of hemostatic activation, and factor VIII, a procoagulant cofactor, indicating a potential mechanism for statin use to lower incidence of venous thromboembolism[6].

Renal Function

Kidney function and injury were assessed using longitudinal measurements of common clinical biomarkers as well as the novel biomarker cystatin C. Cystatin C is a cysteine protease inhibitor secreted by all nucleated cells that is relatively freely filtered at the glomerulus and increasing levels indicate worsening kidney function. A strong non-linear association of age with cystatin C in MESA suggested that kidney function worsens considerably with age even in those without risk factors for kidney disease. In addition, cystatin C was associated with incident chronic kidney disease (CKD) independent of

microalbuminuria and may have a clinical role in identification of individuals with CKD at highest risk for complications. Inverse associations of cystatin C with left ventricular end-diastolic and end-systolic volumes may partially explain the relationship between kidney dysfunction and heart failure. MESA has also made important contributions to understanding racial/ethnic differences in kidney function decline. African-Americans and Hispanics had higher rates of kidney function decline than Whites, while Whites and Chinese-Americans had similar rates of decline[7].

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone hormone system regulates plasma sodium concentration and arterial blood pressure and, therefore, plays major roles in hypertension and atherosclerotic CVD, not only through direct effects on blood pressure, but also through effects on cardiac fibrosis and end-organ damage independent of blood pressure. Prior to MESA, no large studies, outside of clinic cohorts with hypertension, had examined racial/ethnic differences in renin or aldosterone levels. In MESA, there were notable racial/ethnic differences in both analytes, with African-Americans having lower aldosterone levels, and Hispanics having higher plasma renin activity levels, compared to other groups. Further, Hispanics appeared to be more sensitive to both hormones with a stronger association between renin or aldosterone and blood pressure. These findings may have important implications in racial/ethnic differences in the diagnosis and treatment of hypertension and resulting morbidity and mortality[8].

Adipokines and Metabolism

Adipokines, such as leptin, resistin and adiponectin, are cytokines secreted by adipose tissue which influence multiple metabolic pathways. In MESA, higher leptin levels were associated with lower risk of all-cause mortality and CVD. MESA has also contributed to the literature on racial/ethnic differences in circulating levels of adiponectin and leptin and their link to racial/ethnic differences in insulin resistance. Associations of leptin and adiponectin with insulin resistance did not vary significantly among racial/ethnic groups; however, associations of body mass index with adiponectin and leptin differed by significantly by race/ethnicity, suggesting roles for leptin and adiponectin in racial/ethnic differences in insulin resistance[9]. Likewise, while resistin was associated with CVD across all racial/ethnic groups, significant racial/ethnic interactions were noted; the impact of resistin on CVD may be especially important in Hispanics.

Plaque Stability

Lp-PLA_2 is an enzyme responsible for hydrolysis of oxidized phospholipids on LDL particles. Higher Lp-PLA_2 activity within an atherosclerotic plaque is associated with greater vulnerability of the plaque to rupture. Extending previous research to a multi-ethnic cohort, both Lp-PLA_2 mass and activity were associated with increased risk of incident CVD and CHD in MESA, regardless of the presence of subclinical CVD[10].

Matrix metalloproteinase-9 (MMP-9) is an enzyme produced by a number of cellular constituents of atherosclerotic plaques, vessel walls and the myocardium. MMP-9 functions in collagen degradation and tissue remodeling. In MESA, MMP-9 was associated with lower

right ventricular mass in individuals free of clinical CVD potentially through prevention of collagen accumulation[11].

Endothelial Cell Function

Biomarkers of endothelial perturbation include soluble forms of cellular adhesion proteins like intracellular adhesion molecule-1 (sICAM-1) and P-selectin. Higher levels of sICAM-1 were associated with accelerated progression of emphysema in MESA, indicating that neutrophil recruitment to the lung, mediated by ICAM-1, may play a role in the progression of subclinical emphysema[12]. Similarly, higher levels of P-selectin were associated with peripheral arterial disease, suggesting that leukocytes recruitment to sites of vascular injury, mediated by P-selectin, may contribute to progression of peripheral arterial disease.

Oxidative Stress

Measures of oxidative stress include the stress hormone cortisol and catecholamine neurotransmitters (epinephrine, norepinephrine and dopamine). Sex differences in associations of these measures with diabetes were noted in MESA; women with diabetes had higher total diurnal cortisol exposure than non-diabetic women, while urinary catecholamines were significantly lower in men with diabetes compared to non-diabetic men. These findings are intriguing in that they may detect early autonomic neuropathy in diabetic men and subclinical hypothalamic-pituitary-adrenal axis hyperactivity in diabetic women[13].

Oxidative Damage

Oxidized LDL (oxLDL) cholesterol is a biomarker of oxidative damage. Extending previous findings to younger ages and different ethnic groups, oxLDL was associated with CVD risk factors and multiple measures of subclinical CVD, across sex and racial/ethnic groups, in MESA, supporting its role as a biomarker of atherosclerosis initiation and progression[14].

Vitamin and Mineral Metabolism

MESA also explored the role of mineral metabolism in CVD. Insufficient vitamin D may activate the renin-angiotensin-aldosterone system and stimulate atherogenic cytokine expression, leading to atherosclerosis while excess phosphorus, parathyroid hormone (PTH), and fibroblast growth factor-23 (FGF-23) likely promote medial artery calcification, vascular stiffness, and myocardial hypertrophy.

In MESA, serum 25-hydroxyvitamin D (25[OH]D) concentration varied markedly by race/ethnicity. Lower 25(OH)D was associated with increased risk of CHD among Whites and Chinese-Americans, but not among African-Americans and Hispanics. Lower 25(OH)D was also associated with increased risk of incident CAC, but not other measures of subclinical CVD or heart failure[15]. These findings suggested that insufficient 25(OH)D may be a modifiable risk factor for atherosclerotic CHD, but the ascertainment of vitamin D deficiency, or its biologic impact, may vary by race/ethnicity. Ongoing clinical trials are evaluating the effects of vitamin D supplementation on cardiovascular risk.

Higher serum PTH concentration was associated with arterial stiffness, left ventricular hypertrophy, incident hypertension, and heart failure events, without significant heterogeneity by race/ethnicity[16]. Similarly, higher serum FGF-23 concentration was associated with left ventricular hypertrophy, heart failure events, and incident atrial fibrillation[17]. These findings suggested that PTH, FGF-23, and underlying phosphorus excess, may increase risk of heart failure and related clinical outcomes by reducing vascular compliance or promoting myocardial hypertrophy. Medications targeting PTH, FGF-23, and phosphorus are currently being developed and evaluated.

Fetuin-A, a hepatic secretory protein, inhibits arterial calcification *in vitro* by interacting with calcium and phosphorus to increase their solubility and inhibit precipitation. In humans, fetuin-A circulates at relatively high concentrations and may be a marker of arterial calcification. In a subset of MESA participants, fetuin-A levels were inversely associated with CAC severity, independent of traditional cardiovascular risk factors and kidney function[17].

Chronic Infection

A number of chronic infections acquired early in life and not causing obvious illness are implicated in the development and progression of CVD. These pathogens include persistent viruses such as cytomegalovirus and bacterial pathogens such as *Helicobacter pylori*. In MESA, a high antibody response to multiple pathogens was identified as a better marker of inflammation status (levels of circulating inflammation markers) than seropositivity alone. While associated with inflammation, individual pathogens and pathogen burden (number of positive pathogens) were not associated with measures of subclinical atherosclerosis or CAC, suggesting there was no direct link between infectious burden of these pathogens and subclinical atherosclerosis or subclinical CHD. Although not directly associated with CVD, immune responses to chronic infections maybe an important link in the pathway between psychosocial factors and CVD risk as psychosocial determinants were associated with both pathogen burden and immune response in MESA.

Sex Hormones

Levels of endogenous sex hormones were measured to better understand sex differences in CVD and other diseases. In MESA, higher sex hormone binding globulin levels were associated with less atherogenic lipoprotein profiles while higher endogenous estradiol levels were associated with more atherogenic profiles. Testosterone was associated with favorable lipoprotein profiles in men, but not women, while dehydroepiandrosterone had different associations with lipoprotein subclasses in men and women. These findings highlight the potential clinical utility of sex hormones in improving lipoprotein profiles as well as the complexity of their interactions. In addition, associations of testosterone with QT-interval in men, but not in postmenopausal women, may explain differences in QT-interval duration between men and women. Variations in testosterone level may also contribute to population variability in QT-interval duration in men. In men, an androgenic profile was associated with greater carotid distensibility, while the opposite was found in women[18]. Associations of sex hormones with waist-to-hip ratio also implicated sex hormones in sex-related differences in central obesity[19].

Cardiac Function

Natriuretic peptides have become established markers of long-term cardiovascular prognosis in a variety of clinical settings and even among apparently healthy individuals. In MESA, racial/ethnic differences in N-terminal pro B-type natriuretic peptide (NT-proBNP) levels were noted; African- and Chinese-American participants had lower NT-proBNP concentrations compared to Whites and Hispanics. While both cardiac troponin T (cTnT) and NT-proBNP were independently associated with increased risk of CVD and CHD, only NT-proBNP provided prognostic information above and beyond traditional risk factors, improving risk prediction and classification compared to standard risk equations. Change in NT-proBNP levels over several years was also independently associated with CVD regardless of race/ethnicity[20]. NT-proBNP levels in MESA are an integral component of a clinical algorithm developed to predict incident heart failure.

Immune Cell Profiles

While inflammation biomarkers, such as CRP and IL-6, have elucidated the role of innate immunity and inflammation in atherosclerosis, they do not provide information on the roles of specific cell populations that contribute to CVD development and progression. In particular, $CD4^+$ T helper type 1 (Th1) lymphocytes have been studied extensively and implicated as pro-atherogenic while Th2 cells are thought to be anti-atherogenic. Little information on associations of innate and adaptive immune cells with the progression of atherosclerosis is available from epidemiologic cohorts. The MESA Inflammation study evaluated 11 different cellular phenotypes including Th1 and Th2 cells and $CD4^+$ memory and naïve cells.

Results from variability studies demonstrated that these cellular phenotypes were reproducible and generally stable, indicating, for the first time, their suitability for evaluation in epidemiological research[21].

Th1 cell levels were positively associated with IL-6, CAC and common carotid intima media thickness, whereas Th2 cell levels were negatively associated with common carotid intima media thickness. These results were consistent with a pro-atherogenic role for Th1 cells and an anti-atherogenic role for Th2 cells, and is the first demonstration of these relationships in a multi-ethnic population[21]. In addition, the degree of chronic adaptive immune activation, as estimated by higher memory and lower naïve $CD4^+$ cell phenotypes, was associated with subclinical atherosclerosis and type 2 diabetes.

Biomarker Based Algorithm for Coronary Risk Assessment

MESA was the validation cohort for a study incorporating a number of biomarkers into a CHD risk assessment model that demonstrated clinical utility in improving risk prediction in intermediate risk patients[22]. Seven biomarkers from the domains of inflammation, chemotaxis, apoptosis and growth/angiogenesis factors (cutaneous T-cell-attracting chemokine, eotaxin, factor activating Exos ligand (FasLigand), soluble factor activating Exos (sFas), hepatocyte growth factor, interleukin-16 and monocyte chemoattractant protein-3) were included in the model which demonstrated a net clinical reclassification

index of 42.7%, exceeding the clinical net classification indices of established risk factor scores[22].

Participation in Multi-Cohort Consortia

MESA has provided biomarker data to several multi-cohort consortia involved with genomics research including: the Candidate Gene Association Resource (CARe), Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), Population Architecture using Genomics and Epidemiology (PAGE), Exome Sequencing Project (ESP), and most recently, Trans-omics for Precision Medicine (TOPMed) and the Emerging Risk Factor Collaboration (EFRC), a biomarker meta-analysis. Important meta-analyses completed to date include those for fibrinogen and CRP. In addition, MESA has participated in an international metabolomics consortium, the COMBInational BIOmarkers for subclinical atherosclerosis (COMBI-BIO) for which primary data are currently being analyzed.

Summary

Biomarkers are powerful instruments in the examination of the full spectrum of a disease; from initial development, through progression to clinical stages. To date, the MESA parent and ancillary studies have measured over 180 biomarkers covering 26 different biological domains. Additional biomarker measurements, utilizing the MESA repository of plasma, serum and urine samples from each exam, are ongoing. The results of these studies have contributed to, and will continue to contribute to, clinical recommendations utilizing biomarkers for diagnosis and treatment, and a better understanding of racial/ethnic and sex differences in key biological pathways which reflect racial/ethnic and sex differences in disease prevalence and presentation.

Overall, these studies highlight the importance of circulating and urinary biomarkers; they are not merely surrogate endpoints. Biomarkers are able to reflect the entire span of a particular disease from the earliest subclinical manifestations to clinical stages and can broaden our knowledge about the underlying pathogenesis of disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MESA	Multi-Ethnic Study of Atherosclerosis
CVD	Cardiovascular Disease
CHD	Coronary Heart Disease
CKD	Chronic Kidney Disease
CAC	Coronary Artery Calcium
CRP	C-reactive Protein
IL-6	Interleukin-6
HDL	high density lipoprotein
LDL	low density lipoprotein

References

1. Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB Jr. Herrington DM. Gender and c-reactive protein: Data from the Multi-Ethnic Study of Atherosclerosis (MESA) cohort. Am Heart J. 2006; 152:593–8. [PubMed: 16923436]
2. Weiner SD, Ahmed HN, Jin Z, Cushman M, Herrington DM, Nelson JC, Di Tullio MR, Homma S. Systemic inflammation and brachial artery endothelial function in the Multi-Ethnic Study of Atherosclerosis (MESA). Heart. 2014; 100:862–6. [PubMed: 24714919]
3. Leary PJ, Jenny NS, Barr RG, Bluemke DA, Harhay MO, Heckbert SR, Kronmal RA, Lima JA, Mikacenic C, Tracy RP, Kawut SM. Pentraxin-3 and the right ventricle: The Multi-Ethnic Study of Atherosclerosis-right ventricle study. Pulm Circ. 2014; 4:250–9. [PubMed: 25006444]
4. Otvos JD, Mora S, Shalaurova I, Greenland P, Mackey RH, Goff DC Jr. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. J Clin Lipidol. 2011; 5:105–13. [PubMed: 21392724]
5. Guan W, Cao J, Steffen BT, Post WS, Stein JH, Tattersall MC, Kaufman JD, McConnell JP, Hoefner DM, Warnick R, Tsai MY. Race is a key variable in assigning lipoprotein(a) cutoff values for coronary heart disease risk assessment: The Multi-Ethnic Study of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2015; 35:996–1001. [PubMed: 25810300]
6. Adams NB, Lutsey PL, Folsom AR, Herrington DH, Sibley CT, Zakai NA, Ades S, Burke GL, Cushman M. Statin therapy and levels of hemostatic factors in a healthy population: The Multi-Ethnic Study of Atherosclerosis. J Thromb Haemost. 2013; 11:1078–84. [PubMed: 23565981]
7. Peralta CA, Katz R, DeBoer I, Ix J, Sarnak M, Kramer H, Siscovick D, Shea S, Szklo M, Shlipak M. Racial and ethnic differences in kidney function decline among persons without chronic kidney disease. J Am Soc Nephrol. 2011; 22:1327–34. [PubMed: 21700831]
8. Rifkin DE, Khaki AR, Jenny NS, McClelland RL, Budoff M, Watson K, Ix JH, Allison MA. Association of renin and aldosterone with ethnicity and blood pressure: The Multi-Ethnic Study of Atherosclerosis. Am J Hypertens. 2014; 27:801–10. [PubMed: 24436325]
9. Rasmussen-Torvik LJ, Wassel CL, Ding J, Carr J, Cushman M, Jenny N, Allison MA. Associations of body mass index and insulin resistance with leptin, adiponectin, and the leptin-to-adiponectin ratio across ethnic groups: The Multi-Ethnic Study of Atherosclerosis (MESA). Ann Epidemiol. 2012; 22:705–9. [PubMed: 22929534]
10. Garg PK, McClelland RL, Jenny NS, Criqui MH, Greenland P, Rosenson RS, Siscovick DS, Jorgensen N, Cushman M. Lipoprotein-associated phospholipase A_2 and risk of incident cardiovascular disease in a multi-ethnic cohort: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis. 2015; 241:176–82. [PubMed: 26004387]

11. Kawut SM, Barr RG, Johnson WC, Chahal H, Tandri H, Jain A, Bristow MR, Kizer JR, Bagiella E, Lima JA, Bluemke DA. Matrix metalloproteinase-9 and plasminogen activator inhibitor-1 are associated with right ventricular structure and function: The MESA-RV study. Biomarkers. 2010; 15:731–8. [PubMed: 20923324]
12. Aaron CP, Schwartz JE, Bielinski SJ, Hoffman EA, Austin JH, Oelsner EC, Donohue KM, Kalhan R, Berardi C, Kaufman JD, Jacobs DR Jr. Tracy RP, Barr RG. Intercellular adhesion molecule 1 and progression of percent emphysema: The MESA Lung Study. Respir Med. 2015; 109:255–64. [PubMed: 25457724]
13. Champaneri S, Xu X, Carnethon MR, Bertoni AG, Seeman T, Diez Roux A, Golden SH. Diurnal salivary cortisol and urinary catecholamines are associated with diabetes mellitus: The Multi-Ethnic Study of Atherosclerosis. Metabolism. 2012; 61:986–95. [PubMed: 22209664]
14. Holvoet P, Jenny NS, Schreiner PJ, Tracy RP, Jacobs DR. The relationship between oxidized LDL and other cardiovascular risk factors and subclinical CVD in different ethnic groups: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2007; 194:245–52. [PubMed: 16982059]
15. Mathew JS, Leary PJ, Bansal N, Deo R, Lima JA, Siscovick DS, Kestenbaum B, Kawut SM, de Boer IH. Mineral metabolism and the right ventricle: The Multi-Ethnic Study of Atherosclerosis (MESA). Am J Kidney Dis. 2015; 65:521–3. [PubMed: 25453996]
16. Bansal N, Zelnick L, Robinson-Cohen C, Hoofnagle AN, Ix JH, Lima JA, Shoben AB, Peralta CA, Siscovick DS, Kestenbaum B, de Boer IH. Serum parathyroid hormone and 25-hydroxyvitamin D concentrations and risk of incident heart failure: The Multi-Ethnic Study of Atherosclerosis. J Amer Heart Assoc. 2014; 3:e001278. [PubMed: 25468653]
17. Ix JH, Katz R, de Boer IH, Kestenbaum BR, Peralta CA, Jenny NS, Budoff M, Allison MA, Criqui MH, Siscovick D, Shlipak MG. Fetuin-a is inversely associated with coronary artery calcification in community-living persons: The Multi-Ethnic Study of Atherosclerosis. Clin Chem. 2012; 58:887–95. [PubMed: 22377528]
18. Vaidya D, Golden SH, Haq N, Heckbert SR, Liu K, Ouyang P. Association of sex hormones with carotid artery distensibility in men and postmenopausal women: Multi-Ethnic Study of Atherosclerosis. Hypertension. 2015; 65:1020–5. [PubMed: 25753974]
19. Mongraw-Chaffin ML, Anderson CA, Allison MA, Ouyang P, Szklo M, Vaidya D, Woodward M, Golden SH. Association between sex hormones and adiposity: Qualitative differences in women and men in the Multi-Ethnic Study of Atherosclerosis. J Clin Endocrinol Metabol. 2015; 100:E596–600.
20. Daniels LB, Clopton P, deFilippi CR, Sanchez OA, Bahrami H, Lima JA, Tracy RP, Siscovick D, Bertoni AG, Greenland P, Cushman M, Maisel AS, Criqui MH. Serial measurement of N-terminal pro-B-type natriuretic peptide and cardiac troponin T for cardiovascular disease risk assessment in the Multi-Ethnic Study of Atherosclerosis (MESA). Am Heart J. 2015; 170:1170–83. [PubMed: 26678639]
21. Tracy RP, Doyle MF, Olson NC, Huber SA, Jenny NS, Sallam R, Psaty BM, Kronmal RA. T-helper type 1 bias in healthy people is associated with cytomegalovirus serology and atherosclerosis: The Multi-Ethnic Study of Atherosclerosis. J Am Heart Assoc. 2013; 2:e000117. [PubMed: 23688675]
22. Cross DS, McCarty CA, Hytopoulos E, Beggs M, Nolan N, Harrington DS, Hastie T, Tibshirani R, Tracy RP, Psaty BM, McClelland R, Tsao PS, Quertermous T. Coronary risk assessment among intermediate risk patients using a clinical and biomarker based algorithm developed and validated in two population cohorts. Curr Med Res Opin. 2012; 28:1819–30. [PubMed: 23092312]

Highlights

- Multi-Ethnic Study of Atherosclerosis has measured over 130 circulating and urinary biomarkers of inflammation and other novel and emerging specific biological pathways
- Pathways include inflammation, insulin resistance, lipids and fatty acids, hemostasis and fibrinolysis, renal function, renin-angiotensin-aldosterone system, adipokines, atherosclerotic plaque stability, endothelial cell function, oxidative damage, vitamins and minerals, chronic infection, sex hormones, cardiac function and immune cell profiles
- These biomarkers have contributed to over 1000 research articles and many clinical recommendations
- Biomarker measurements are also being utilized in multi-cohort consortia examining genomics, trans-omics and metabolomics

Table 1

Biomarkers measured in MESA by domain (biological pathway)

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	**Biomarker**	**1**	**2**	**3**	**4**	**5**
Inflammation	C-reactive protein (high sensitivity)	6762		1935	442	501
	Interleukin-6	6622		1923		
	Fibrinogen antigen	6767		1967	456	517
	White blood cell count				928	2892
	Interleukin-10	2810		866		
	Interleukin-2 soluble receptor	2885				
	Tumor necrosis factor-α soluble receptor 1	2885	2372			
	Pentraxin-3	2838				
	Serum amyloid P	2863				
	Anti-human heat shock protein-60	998				
	Interleukin-16	824				
	Macrophage migration inhibitory factor	824				
	Macrophage inflammatory protein-1α	824				
	Myeloperoxidase	824				
	Tumor necrosis factor-α		779	1182		
Insulin Resistance	Fasting glucose	6789	6184	5887	5634	4587
	Fasting insulin	6784		1965		
	Hemoglobin A_1C		6142			
Lipids/Fatty Acids	Total cholesterol, HDL cholesterol, LDL cholesterol	6791	6185	5892	5634	4582
	Triglycerides	6971	6185	5892	5634	4582
	Small HDL 7.3-8.2 nm from NMR[#]	6795				
	Medium HDL 8.2-8.8 nm from NMR[#]	6795				
	HDL cholesterol (total) from NMR[#]	6795				

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
	HDL particles (total) from NMR[#]	6795				
	Mean HDL size from NMR[#]	6795				
	Large HDL 8.8-13 nm from NMR[#]	6795				
	Large HDL 9.4-14 nm from NMR[#]	6786				
	Large LDL 20.5-23 nm from NMR[#]	6786				
	Large LDL 21.2-23 nm from NMR[#]	6795				
	Large VLDL > 60 nm from NMR[#]	6786				
	LDL (total) 18-21.2 from NMR[#]	6795				
	Very small LDL 18-19.8 nm from NMR[#]	6786				
	Small LDL 18-20.5 nm from NMR[#]	6795				
	Medium-small LDL 19.8-21.2 nm from NMR[#]	6795				
	LDL particles (total) from NMR[#]	6795				
	Mean LDL size from NMR[#]	6795				
	Total triglycerides from NMR[#]	6786				
	Small 27-35 nm VLDL from NMR[#]	6795				
	Medium 35-60 VLDL from NMR[#]	6795				
	VLDL triglycerides (total) from NMR[#]	6795				
	VLDL particles (total) from NMR[#]	6795				
	Mean VLDL size from NMR[#]	6795				
	HDL subfractions (HDL-1, -2, -3, -4, -5, -6, -7, -8)	997				
	Remnant-like particle cholesterol	999				
	Cholesterol ester transferase protein activity	982				

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
	Cholesterol ester transferase protein mass	999				
	Sphingomyelin	6708				
	Apolipoprotein A1	4679				
	Apolipoprotein B	4676				
	Lipoprotein(a)	4676				2892
	Free fatty acid	6723				
	14:0 Myristic acid	2856				
	15:0 Pentadecanoic acid	2856				
	16:0 Hexadecanoic acid	2856				
	16:1 9 Cis Palmitoleic acid	2856				
	16:1 9 Trans Palmitoleic acid	2856				
	18:0 Stearic acid	2856				
	18:1 12 Trans Oleic acid	2856				
	18:1 9-11 Trans Oleic acid	2856				
	18:1 Cis Linoleic acid	2856				
	18:1 12 Cis Linoleic acid	2856				
	18:1 9 Cis Linoleic acid	2856				
	18:2 C/C Linoleic acid	2856				
	18:2 C/T Linoleic acid	2856				
	18:2 T/C Linoleic acid	2856				
	18:2 T/T Linoleic acid	2856				
	18:3 N3 α-Linoleic acid	2856				
	18:3 M6 γ-Linoleic acid	2856				
	20:0 Arachidonic acid	2856				
	20:1 N9 Gadoleic acid	2856				
	20:2 N6 Eicosanoic acid	2856				
	20:3 N6 Eicosanoic acid	2856				

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	**Biomarker**	**1**	**2**	**3**	**4**	**5**
	20:4 N6 Arachidonic acid	2856				
	20:5 N3 Timnodonic acid	2856				
	22:0 Behenic acid	2856				
	22:5 N3 Clupanodonic acid	2856				
	22:6 N3 Docosahexaenoic acid	2856				
	24:1 N9 Nervonic acid	2856				
Hemostasis/Fibrinolysis	D-dimer	6769			456	515
	Factor VIII activity	6765				
	Plasminogen activator inhibitor-1	973				
	Plasmin-antiplasmin complex	6627				
	Thrombin activatable fibrinolysis inhibitor					
	Tissue factor pathway inhibitor	995				
Oxidative Damage	Oxidized LDL cholesterol	999				
	F_2-isoprostanes	390				
Oxidative Stress	Salivary cortisol (measured across exams 3 and 4)			1002		
	Urinary epinephrine* (measured across exams 3 and 4)			1002		
	Urinary norepinephrine* (measured across exams 3 and 4)			1002		
	Urinary dopamine* (measured across exams 3 and 4)			1002		
Renal Function	Serum creatinine	6789	769	5887	5634	4587
	Cystatin C	6756	770	5550	5260	4581
	Urine Creatinine/Albumin*	6789	769	5887	5634	4587
	Blood urea nitrogen*	6738				
	Kidney injury molecule-1*	686				
	Neutrophil gelatinase-associated lipocalin*	686				
Renin-Angiotensin-Aldosterone System	Plasma renin activity		698	1103		

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Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
	Aldosterone		732	1158		
Adipokines/Metabolism	Leptin (measured across exams 2 and 3)	824	1960			
	Adiponectin (measured across exams 2 and 3)	824	1967			
	Resistin (measured across exams 2 and 3)	824	1966			
Plaque Stability	Lipoprotein phospholipase A_2 mass	5273				
	Lipoprotein phospholipase A_2 activity	5353				
	Matrix metalloproteinase-3	999				
	Matrix metalloproteinase-9	999				
	Matrix metalloproteinase-1		2372			
	Matrix metalloproteinase-2		2372			
	CD40 ligand	999				
	Soluble tissue factor	993				
Vitamin/Mineral	24,25-Dihydroxy vitamin D3	6473				
	1,25-Dihydoxy vitamin D2	440				
	25-Hydroxy vitamin D		368			
	Parathyroid hormone	6555				
	Fetuin-A	2904				
	Fibroblast growth factor-23	6552				
	Serum calcium	6514				
	Serum chloride	6489				
	Serum phosphorus	6544				
	Serum sodium	6489				
	Serum bicarbonate	6489				
	Serum potassium	6489		650	323	
	Dihydrophylloquinone	1056				
	Phylloquinone	1056				
Endothelial Cell Function	Homocysteine	6794				

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	**Biomarker**	**1**	**2**	**3**	**4**	**5**
	von Willebrand factor	2885				
	Soluble intracellular adhesion molecule-1	2622	2372		455	512
	Soluble thrombomodulin	997				
	Soluble E-selectin	999			455	516
	Soluble P-selectin	5974	2372			
	Soluble L-selectin		2372			
	Soluble vascular cell adhesion molecule-1		2372			
	Chemokine ligand 21		2372			
	E-cadherin		2372			
	Regulated on Activation, Normal T Expressed and Secreted (RANTES)	824	2372			
	Stromal derived factor 1α		2372			
	Secretory leukocyte protease inhibitor		2372			
	Transforming growth factor β1		2372			
	Tissue inhibitor of metalloproteinases 2		2372			
	Endothelial progenitor cells (count per 10,000 lymphocytes)				407	
Growth Factors/	Hepatocyte growth factor	5974				
Angiogenesis	Angiopoietin-2	824				
	Epidermal growth factor-1	824				
	Vascular endothelial growth factor (VEGF)	824				
Chemotaxis	Cutaneous T-cell-attracting chemokine (CCL27)	824				
	Eotaxin-3	824				
	Interferon-inducible T-cell alpha chemoattractant (CXCL11)	824				
	Interferon gamma-induced protein-10 (IP-10)	824				
	Monocyte chemoattractant protein-1	824				
	Monocyte chemoattractant protein-2	824				
	Monocyte chemoattractant protein-3	824				

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	**Biomarker**	**1**	**2**	**3**	**4**	**5**
	Monocyte chemoattractant protein-4	824				
Chronic Infection	*Chlamydia Pneumoniae* (antibody titer)	6790				
	Cytomegalovirus (antibody titer)	999				
	Helicobacter Pylori (antibody titer)	999				
	Hepatitis A virus (antibody titer)	999				
	Herpes Simplex virus (antibody titer)	999				
Cardiac Function	N-terminal pro-brain natriuretic peptide	5597		4694		
	Cardiac troponin T	5597		4694		
Apoptosis	Factor activating Exos ligand (FasLigand)	824				
	Soluble factor activating Exos (Fas)	824				
Macrophage Activity	Interleukin-18	824				
Vascular Remodeling	Tissue inhibitor of metalloproteinase-1 (TIMP-1)	824				
	Tissue inhibitor of metalloproteinase-4 (TIMP-4)	824				
Sex Hormones	Dehydroepiandrosterone	6172				
	Sex hormone binding globulin	6172				
	Testosterone	6167				
	Estradiol	6170				
Immune Cell Profiles	% $CD4^+$ lymphocytes that are Th1 cells				916	
	% $CD4^+$ lymphocytes that are Th2 cells				917	
	% $CD4^+$ lymphocytes that are memory cells				917	
	% $CD4^+$ lymphocytes that are naïve cells				917	
	% cell that are monocytes - LPS stimulated expression assay				842	
	% cell that are monocytes - unstimulated expression assay				843	
	Immature granulocytes					1645
Autoimmunity	Rheumatoid factor IgA	6738				
	Rheumatoid factor IgM	6738				

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Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	**Biomarker**	**1**	**2**	**3**	**4**	**5**
Bone Morphology	Osteoprotegerin		761			
Liver Function	Gamma-glutamyltransferase	6754				
Blood Oxygenation	Red blood cells				928	2892
	Hemoglobin				927	2892
	Platelets				922	2886
Tobacco Smoke Exposure	Urinary cotinine*					3212

*Measured in urine. All other biomarkers measured in serum or plasma unless otherwise noted.

#from NMR Lipoprofile-II spectral analysis.