SUPPLEMENTAL MATERIAL

Supplementary Methods

Clinical Examination and Laboratory Assays.

MDC participants underwent baseline history, examination, and laboratory assessment. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure (≥ 90 mmHg, or use of antihypertensive therapy. Glucose was measured in venous whole blood. Diabetes was defined as fasting blood glucose ≥ 6.1 mmol/L (corresponding to fasting plasma glucose of ≥ 7.0 mmol/L or 126 mg/dL), a self-reported physician diagnosis of diabetes, or use of anti-diabetic medication. Cigarette smoking was elicited by a self-administered questionnaire, with current cigarette smoking defined as any use within the past year.

Fasting EDTA plasma was frozen at −80°C immediately after collection. CysC was analyzed using a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Deerfield, Illinois).[1-2] Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) Study equation.[3] Fasting levels of HDL-C and TG were available in 4662 and 4709 of the 4757 individuals with baseline cysC and were measured according to standard procedures at the University Hospital Malmö. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as [fasting insulin (μU/ml) x fasting glucose (mmol/l)] / 22.5 as previously described[4] and was available in 4581 individuals.

Total and differential peripheral leukocytes were counted using a SYSMEX K1000 automatic cell counter (Sysmex Europe, Norderstedt, Germany). The analyses were performed at the

time of the screening examination, at the University Hospital Malmö, using fresh heparinized blood. The leukocyte histogram was discriminated into small, middle, and large sized leukocytes by a three-part differential method, providing the following information in 1 uL of whole blood: absolute count of lymphocytes (small cells), absolute count of monocytes plus basophils/eosinophils (middle sized cells), and absolute count of neutrophils (large cells).[5] The baseline count of middle sized cells consists mainly of monocytes,[5] and in line with smaller studies linking monocytes to atherosclerosis[6-7] and CV events,[8] it has recently been shown to predict future coronary events in a large adjusted analysis of over 25,000 individuals.[5] We shall refer to this count as monocytes, given the relative scarcity of basophils/eosinophils compared to monocytes in this count.[5] Monocytes were available in all 4757 MDC-CC individuals with baseline cysC.

Metabolite Profiling. Plasma samples were stored at -80˚ C and profiled using liquid chromatography-tandem mass spectrometry (LC-MS) as described previously.[9-10] LC-MS was performed using a 4000 QTRAP triple quadrupole mass spectrometer (AB SCIEX, Foster City, CA), coupled to a multiplexed LC system including two 1200 Series pumps (Agilent Technologies, Santa Clara, CA) and an HTS PAL autosampler (Leap Technologies, Carrboro, NC) with 2 injection ports and a column selection valve. The two pumps were similarly configured for hydrophobic interaction chromatography (HILIC) using 150 x 2.1 mm Atlantis HILIC columns (Waters; Milford, MA). MultiQuant software v1.1 (AB SCIEX, Foster City, CA) was used for automated peak integration and metabolite peaks also were manually reviewed for quality of integration. Formic acid, ammonium acetate, LC-MS grade solvents, and valine-d8 were obtained from Sigma-Aldrich (St. Louis, MO), with the remainder of isotopically-labeled analytical standards obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). Internal standard peak areas were monitored for quality control and individual samples with

peak areas differing from the group mean by more than 2 standard deviations were re-analyzed. A complete list of metabolites analyzed is given in the sub-text of Table 5. Metabolites were selected based on the following criteria: 1) known structural identity; 2) distribution across multiple biochemical pathways; 3) reliable measurement using LC-MS in a high throughput fashion; and, 4) low rate of missingness on our platform (<1%). Using sample preparation and MS replicates of human samples, we have previously documented coefficients of variation (CV) for the metabolites in the platform: 54% of metabolites have CV ≤ 10% and 74% have CV ≤ 20%.[9]

Clinical Endpoints

We primarily examined the top quartile of monocytes, measured at the time of the screening exam and defined above. We examined a secondary endpoint at baseline: maximal carotid bulb IMT (IMT_{max}Bulb). Using B-mode ultrasound, the right carotid bifurcation was scanned within a pre-defined "window" comprising 3 cm of the distal common carotid artery, the bifurcation, and 1 cm of the internal and external carotid artery, respectively. The maximum thickness of the intima–media (max IMT bifurcation) in the far wall of the carotid bifurcation was measured off-line according to the leading edge principle, using a specially designed computer-assisted image analyzing system as previously described.[11] IMT_{max}Bulb (measured in millimeters) was available in 3134 MDC-CC participants with available covariate data.

References for Supplementary Methods

1. Shlipak MG, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, Siscovick DS, Stehman-Breen C. Cystatin c and the risk of death and cardiovascular events among elderly persons. *N Engl J Med*. 2005;352:2049-2060

2. Shlipak MG, Katz R, Sarnak MJ, Fried LF, Newman AB, Stehman-Breen C, Seliger SL, Kestenbaum B, Psaty B, Tracy RP, Siscovick DS. Cystatin c and prognosis for cardiovascular and kidney outcomes in elderly persons without chronic kidney disease. *Ann Intern Med*. 2006;145:237-246
3. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med*. 1999;130:461-470
4. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: Studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*. 2000;23:57-63
5. Adamsson Eryd S, Smith JG, Melander O, Hedblad B, Engstrom G. Incidence of coronary events and case fatality rate in relation to blood lymphocyte and neutrophil counts. *Arterioscler Thromb Vasc Biol*. 2012;32:533-539
6. Chapman CM, Beilby JP, McQuillan BM, Thompson PL, Hung J. Monocyte count, but not c-reactive protein or interleukin-6, is an independent risk marker for subclinical carotid atherosclerosis. *Stroke*. 2004;35:1619-1624
7. Johnsen SH, Fosse E, Joakimsen O, Mathiesen EB, Stensland-Bugge E, Njolstad I, Arnesen E. Monocyte count is a predictor of novel plaque formation: A 7-year follow-up study of 2610 persons without carotid plaque at baseline the tromso study. *Stroke*. 2005;36:715-719
8. Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, Ulrich C, Fliser D, Heine GH. Cd14++cd16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. *Eur Heart J*. 2011;32:84-92

9. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, O'Donnell CJ, Carr SA, Mootha VK, Florez JC, Souza A, Melander O, Clish CB, Gerszten RE. Metabolite profiles and the risk of developing diabetes. *Nat Med*. 2011;17:448-453

10. Cheng S, Rhee EP, Larson MG, Lewis GD, McCabe EL, Shen D, Palma MJ, Roberts LD, Dejam A, Souza AL, Deik AA, Magnusson M, Fox CS, O'Donnell CJ, Vasan RS, Melander O, Clish CB, Gerszten RE, Wang TJ. Metabolite profiling identifies pathways associated with metabolic risk in humans. *Circulation*. 2012;125:2222-2231

11. Hedblad B, Wikstrand J, Janzon L, Wedel H, Berglund G. Low-dose metoprolol cr/xl and fluvastatin slow progression of carotid intima-media thickness: Main results from the beta-blocker cholesterol-lowering asymptomatic plaque study (bcaps). *Circulation*. 2001;103:1721-1726