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). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) Study equation. 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 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). The baseline count of middle sized cells consists mainly of monocytes, and in line with smaller studies linking monocytes to atherosclerosis and CV events, it has recently been shown to predict future coronary events in a large adjusted analysis of over 25,000 individuals. We shall refer to this count as monocytes, given the relative scarcity of basophils/eosinophils compared to monocytes in this count. 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. 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%.

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_{maxBulb}). 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. IMT_{maxBulb} (measured in millimeters) was available in 3134 MDC-CC participants with available covariate data.

References for Supplementary Methods


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


