

Study	Biomarker Measurement
<b>ARIC</b>	<p>Frozen fasting blood samples were obtained at the baseline examinations and stored at -70°C.</p> <p><i>MCP-1:</i> Plasma concentrations of MCP-1 were measured in duplicate by direct sandwich ELISA (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA <a href="http://www.gelifesciences.co.jp/tech_support/manual/pdf/cellasy/RPN2769.pdf">http://www.gelifesciences.co.jp/tech_support/manual/pdf/cellasy/RPN2769.pdf</a>.) and the intra-assay coefficient of variation was 5.78%.</p> <p><i>RANTES:</i> RANTES was measured in by ELISA (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA <a href="http://www6.gelifesciences.com/aptrix/upp00919.nsf/Content/ECB90528BB441B9CC1256FAE000DC2B5/\$file/RPN5964PL_Rev_B_02-2008_WEB.pdf">http://www6.gelifesciences.com/aptrix/upp00919.nsf/Content/ECB90528BB441B9CC1256FAE000DC2B5/\$file/RPN5964PL_Rev_B_02-2008_WEB.pdf</a>) The minimal detectable dose was 3.5 pg/mL, the standard curve ranged from 0-1000 pg/mL. Intra- and interassay coefficients of variation were 7.7% and 8.5% respectively. The coefficient of variation for re-measurement was 20%.</p>
<b>FHS</b>	<p>Fasting blood samples were obtained from participants, processed promptly, centrifuged and frozen at -80°C. All samples were run in duplicate and averaged.</p> <p><i>Intercellular adhesion molecule-1:</i> Intercellular adhesion molecule-1 was measured in serum using a commercially available ELISA from R &amp; D Systems (Cat. No. BBE 1B) <a href="http://www.rndsystems.com/">http://www.rndsystems.com/</a>. Minimum detectable dose: &lt;0.35 ng/ml; standard curve range: 0-50 ng/mL. The intra-assay coefficient of variation was 3.7%±2.4, The coefficient of variation threshold for re-measurement was 8.8%.</p> <p><i>Interleukin-6:</i> Interleukin-6 was measured in serum using a commercially available ELISA from R &amp; D Systems (Cat. No. D6050) <a href="http://www.rndsystems.com/">http://www.rndsystems.com/</a>. Minimum detectable dose: &lt;0.70 pg/mL; Standard curve range: 0–300 pg/mL. The intra-assay coefficient of variation was 3.1%±2.2. The coefficient of variation threshold for re-measurement was 7.9%.</p> <p><i>MCP-1:</i> MCP-1 was measured in serum (n=6771) and EDTA plasma (n=263) using a commercially available ELISA from R&amp;D Systems (Cat. No. DCP00) <a href="http://www.rndsystems.com/">http://www.rndsystems.com/</a>.<sup>15</sup> Minimum detectable dose: &lt;5.0 pg/mL; Standard curve range: 0–2000 pg/mL. The intra-assay coefficient of variation was 3.8%±3.3. The coefficient of variation threshold for re-measurement was 13.1%.</p> <p><i>Myeloperoxidase:</i> Myeloperoxidase was measured in serum using a commercially available ELISA from Oxis (Cat. No. 21013) <a href="http://www.oxis.com/">http://www.oxis.com/</a>. Minimum detectable dose: 0.17 ng/mL; Standard curve range: 0–25 ng/mL. The intra-assay coefficient of variation was 3.0%±2.5. The coefficient of variation threshold for re-measurement was 11.5%.</p>
<b>MONICA/ KORA</b>	<p>A non-fasting venous blood sample was obtained from all study participants while sitting at baseline. The blood was centrifuged at 3,000 g for 10 minutes within 30 min after venipuncture, immediately aliquoted and frozen at -80°C until further analysis.</p> <p><i>C-reactive protein:</i></p>

Study	Biomarker Measurement
	<p>CRP was measured in serum by a high-sensitive immunoradiometric assay (IRMA) using a 5-point calibration with the WHO international reference standard 85/50637 (range, 0.05-10 mg/L). The intra- and inter-assay coefficients of variation of quality control test sera for CRP were 4.0 and 12.0%.</p> <p><i>Intercellular adhesion molecule-1:</i>  Intercellular adhesion molecule-1 was measured in serum using a commercially available ELISA (Diacclone, Besancon, France). The intra- and inter-assay coefficients of variation were 2.3 and 4.7%.</p> <p><i>Interferon-inducible protein-10:</i>  Interferon-inducible protein-10 was measured in serum by Luminex multiplex technology using a Luminex 100 analyser (Luminex Corporation, Austin, TX, USA). The intra- and inter-assay CV values of quality control test sera were &lt;10.0 and 35.1%.</p> <p><i>Interleukin-6:</i>  Interleukin-6 was measured in serum using a commercially available ELISA (CLB, Amsterdam, Netherlands). The intra- and inter-assay coefficients of variation of quality control test sera for IL-6 &lt;10.0 and &lt;10.0%.</p> <p><i>Interleukin-8:</i>  Interleukin-8 was measured in serum by Luminex multiplex technology using a Luminex 100 analyser (Luminex Corporation, Austin, TX, USA). The intra- and inter-assay CV values of quality control test sera were &lt;10.0 and 10.9%.</p> <p><i>Interleukin-18:</i>  Interleukin-18 was measured in serum by Luminex technology using an antibody pair and recombinant IL-18 protein from MBL (Nagoya, Japan). The lower detection limit of IL-18 in this assay was approximately 9.8 pg/mL. The intra- and inter-assay coefficients of variation of quality control test sera for IL-18 were &lt;10.0 and &lt;10.7%.</p> <p><i>Macrophage migration inhibitory factor:</i>  Macrophage migration inhibitory factor (MIF) was measured in serum with the Quantikine ELISA kit (R&amp;D Systems, Wiesbaden, Germany). Intra- and inter-assay variability were determined using 3 controls with recombinant MIF and 3 sera in duplicates on 43 plates. Mean intra- and inter-assay CVs were 2.6% and 5.1%, respectively, for the recombinant controls, and 3.8% and 11.1%, respectively, for the sera.</p> <p><i>MCP-1:</i>  For the MONICA/KORA subcohort, MCP-1 was measured in serum by Luminex multiplex technology using a Luminex 100 analyser (Luminex Corporation, Austin, TX, USA). The intra- and inter-assay CV values of quality control test sera were &lt;10.0 and 19.9%. For MONICA/KORA 500k study, MCP-1 was measured in plasma and serum by the Quantikine ELISA kit (R&amp;D Systems, Wiesbaden, Germany). For plasma mean inter-assay coefficient of variation (CV) values was 8.9 %. For serum mean inter-assay coefficient of variation (CV) values was 8.2 %. The correlation coefficient between both assays was 0.579 for 1,623 subjects with higher concentrations for the Luminex assay as has been reported in the literature.<sup>16</sup></p> <p><i>Myeloperoxidase:</i></p>

<b>Study</b>	<b>Biomarker Measurement</b>
	Myeloperoxidase was measured in serum using a commercially available ELISA from Merck (Cat. No. 10-1176-01) <a href="http://www.merck.com/">http://www.merck.com/</a> . Minimum detectable dose: 3 ng/mL; Standard curve range: 0 – 300 ng/mL. 15% of the samples were run in duplicate. The inter-assay coefficient of variation was 10.3%.