Supplementary Data

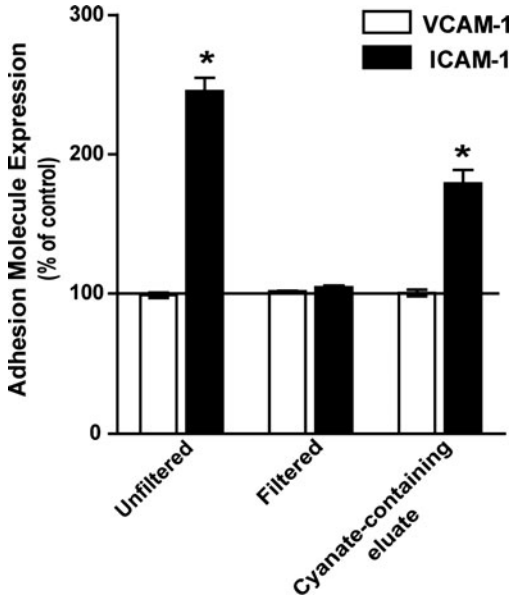
Supplementary Materials and Methods

Reagents

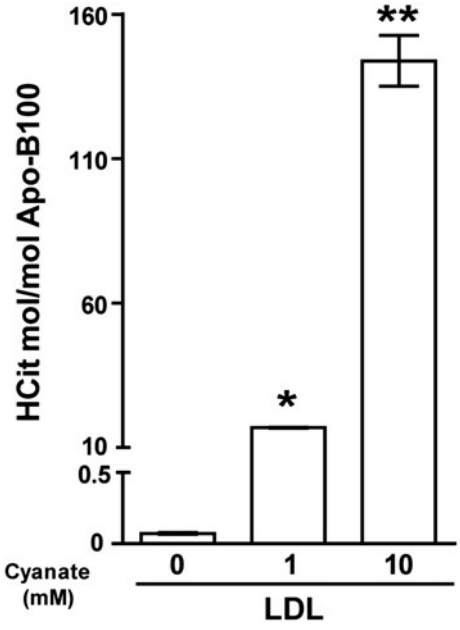
All laboratory reagents were from Sigma unless otherwise specified. The inhibitors of c-Jun N-terminal kinase (JNK-2 inhibitor and JNK-8 inhibitor), p38 mitogen-activated protein kinase (SB203580 and SB202190), extracellular signal-regulated kinase 1/2 (PD98059 and U0126), and nuclear factor-kappa (BAY-11 7082) were purchased from Merck. Inhibitors were dissolved in dimethyl sulfoxide and further diluted to produce a final concentration of the solvent of less than 0.1%. $^{13}C_6$-Homocitrulline was purchased from Ascent Scientific, $^{13}C_6$-lysine from Cambridge Isotope Laboratories, and carboxymethyl-lysine from PolyPeptide Labs. FITC anti-human CD106 (vascular cell adhesion molecule-1), PE anti-human CD54 (intercellular cell adhesion molecule-1), PE anti-human CD62E (E-selectin), FITC mouse IgG1 isotype control, PE mouse IgG1 isotype control, CellFix, and FACS-Flow were from BD Bioscience. Fixative solution was prepared by mixing distilled water (9 mL), FACS-Flow (30 mL), and CellFix (1 mL). The fluorescent dye, calcein-AM, was purchased from Molecular Probes.

Antibodies for immunohistochemistry included anti-mouse CD54 (ICAM-1) from Abbiotec and non-immune rabbit IgG from Lab Vision.

Sodium cyanate was tested for endotoxin by using the Limulus Amoebocyte Lysate assay (25). Endotoxin levels of sodium cyanate used (1 mg/mL) were less than 0.03 EU/mL.



SUPPLEMENTARY FIG. S1. Carbamylated (lipo)proteins do not induce intercellular cell adhesion molecule-1 (ICAM-1) expression in human coronary artery endothelial cells (HCAEC). Growth medium incubated with sodium cyanate was gel filtered on Sephadex PD-10 columns to remove cyanate. Subsequently, the cyanate-containing eluate was collected. HCAEC were then treated for 48 h with unfiltered medium, filtered medium, and the cyanate-containing eluate. Adhesion molecule expression was determined by flow cytometry. All experiments were performed in duplicate. Control was set at 100%, and values are expressed as % of control. Results shown are mean ± SEM ($n = 3$). *$p < 0.05$ *versus* control.



SUPPLEMENTARY FIG. S2. Quantification of carbamyllysine (homocitrulline [HCit]) content in cyanate-treated low-density lipoprotein (LDL) preparations. The carbamyllysine content in LDL exposed to cyanate for 48 h was quantified by liquid chromatography tandem mass spectrometry. Results shown are mean ± SEM ($n = 3$). *$p < 0.05$; **$p < 0.001$ *versus* control.