Introduction

Two members of the lipase family, lipoprotein lipase (LPL) and hepatic lipase (HL) are known to influence plasma lipoprotein metabolism and risk of atherosclerosis in humans [1–5]. Endothelial lipase (EL) is a more recently discovered member of this family of lipases [6,7]. In contrast to LPL and HL, it is expressed in endothelial cells and, in the majority of reports, has relatively more phospholipase activity than the other two enzymes. Similar to HL and LPL, it is secreted and binds to the endothelial surface. In vitro, EL effectively hydrolyzes high-density lipoprotein (HDL) phospholipids. In mice, EL has a major influence on HDL metabolism. Adenovirally mediated overexpression of human EL in mice dramatically reduced HDL cholesterol (HDL-C) concentrations [6], shown to be due to rapid catabolism of HDL [8]. Transgenic overexpression of human EL was shown to reduce HDL-C concentrations as well [9]. Conversely, antibody inhibition of EL in mice significantly increased HDL-C concentrations [10], and EL knockout mice have a significant increase in HDL-C concentrations [9,11]. Furthermore, EL knockout mice crossed onto the apolipoprotein E knockout background have decreased atherosclerosis [12]. Based on these studies in mice, it has been suggested that EL may be an important risk factor and may affect the development of atherosclerosis in humans.

The relationship of EL to variation in lipoprotein concentrations and atherosclerosis in humans has not been reported. Several genetic association studies have suggested that rare variants and common polymorphisms in the human EL gene might be associated with variation in HDL-C concentrations [11,13,14], but they have not been conclusive and were not accompanied by direct measurement of EL concentrations. We hypothesized that concentrations of EL are inversely associated with HDL-C concentrations and directly associated with the metabolic syndrome and atherosclerosis in humans. To test this, we developed an immunoassay for human EL and used it to measure EL mass concentrations in both pre-heparin and post-heparin plasma samples in individuals enrolled in the Study of the Inherited Risk of Atherosclerosis (SIRCA). We determined the association of EL concentrations with lipoprotein concentrations, other cardiovascular risk factors and coronary artery calcification (CAC), a noninvasive measure of coronary atherosclerosis [15–17].

Methods

Study Protocol

SIRCA is a cross-sectional study of asymptomatic individuals and their families designed to investigate novel biomarkers and genetic factors associated with coronary atherosclerosis. The study design and initial findings have previously been published [18,19]. Briefly, persons were eligible for SIRCA if they had a family history of premature coronary artery disease (CAD), were free of clinical CAD, and were men, 20–75 years of age or women, 30–75 years. Exclusion criteria included other major CAD risk factors: known diabetes, total cholesterol higher than 300 mg/dl, cigarette smoking of one pack or more per day, or blood pressure higher than 160/100 mm Hg. The University of Pennsylvania Institutional Review Board approved the study protocol. Informed consent was obtained from each participant. This report focuses on 858 random unrelated individuals from the SIRCA in whom plasma EL concentrations were measured.

Evaluated Parameters

Participants were assessed at the General Clinical Research Center in the University of Pennsylvania Medical Center after a 12-h overnight fast. A questionnaire was administered; height, weight, waist circumference, and blood pressure were obtained; and 30 ml of whole blood was drawn ("pre-heparin" plasma) into EDTA-containing tubes. In a subset of individuals ($n = 510$), blood was drawn 10 min after intravenous administration of heparin (60 units per kilogram body weight). After the blood was centrifuged, the plasma was removed and stored at −80 °C until use ("post-heparin" plasma). Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were measured enzymatically on a Cobas Fara II (Roche Diagnostic Systems, Indianapolis, Indiana, United States) using Sigma reagents (Sigma, St. Louis, Missouri, United States) in a Centers for Disease Control-standardized lipid laboratory. LDL cholesterol was calculated using the Friedewald formula.

Post-heparin plasma samples in EDTA were analyzed for lipoprotein subclasses by nuclear magnetic resonance (NMR) [20–22] (LipoSciences, Raleigh, North Carolina, United States). The NMR method uses the characteristic proton signals broadcast by lipoprotein subclasses to determine both the size and the quantity of each size of lipoprotein. A summary of the reporting format can be found at http://www.liposcience.com. The ten subclasses (and their size ranges) routinely used are: large very low-density lipoprotein (VLDL) (60–200 nm), intermediate VLDL (35–60 nm), small VLDL (27–35 nm), IDL (23–27 nm), large low-density lipoprotein (LDL) (21.3–23 nm), intermediate LDL (19.8–21.2 nm), small LDL (18.3–19.7 nm), large HDL (8.8–13.0 nm), intermediate HDL (8.2–8.8 nm), and small HDL (7.3–8.2 nm).

Electron beam computed tomography was performed on an Imatron C-150 XP/LXP Evolution scanner (Imatron, San Francisco, California, United States) connected to a MagicView workstation for volumetric image reconstruction. Using ECG triggering with the individual in a supine position, 40 contiguous, 3 mm-thick axial slices were acquired just below the carina through the apex of the heart. Global CAC scores were determined according to the method of Agatston [23].

Sandwich Enzyme-Linked Immunosorbent Assay for Human EL

Detailed information on the development and quality control of the sandwich ELISA can be found in Protocol S1. The wells of a 96-well microtiter plate were coated with rabbit anti-human EL antibody. Various concentrations of purified recombinant human EL in phosphate-buffered saline containing 1% BSA were added to the wells as a standard. Plasma samples were diluted 1:10 in phosphate-buffered saline and applied to the wells. Specifically bound protein was incubated with biotin-conjugated rabbit anti-human EL antibody, followed by streptavidin-horseradish peroxidase conjugate, and detection with o-phenylenediamine. The reaction was stopped with 2.5 M sulfuric acid and the plate read at 490 nm. A standard curve of 490 nm absorbance versus the known