concentrations of EL was constructed. The concentration of the plasma samples was determined by comparison to the standard curve multiplied by the dilution factor. Using this ELISA, EL mass concentrations were quantified in plasma collected in the routine manner ("pre-heparin" plasma) in the full sample ($n = 858$) as well as in the post-heparin plasma samples described above ($n = 510$).

Statistical Analysis

Data are reported as median and interquartile range (IQR) or mean ± standard deviation for continuous variables and as proportions for categorical variables. The distributions of both pre-heparin and post-heparin EL mass concentrations were highly skewed rightward, so analyses were performed on log-transformed data. Variables were determined to be normally distributed using the Shapiro-Wilk test. Spearman correlations of EL mass with cardiovascular risk factors and with concentrations of lipoprotein particles, measured by NMR, are presented. The association of EL mass with categorical variables was examined using Kruskal-Wallis rank test. Data were analyzed in the total group and in men and women separately, because the distributions of CAC, HDL, EL, and many risk factors vary with gender [24,25]. Differences in log-transformed EL mass by gender, and cut-points in waist circumference and other risk factors were measured by t-test. Median CAC scores were compared across quartiles of plasma EL (pre-heparin: 6–268, 268.5–422, 422.5–641, and 641.5–2,043; post-heparin: 102–888, 888.5–1,313, 1,313.5–1,927, and 1,927.5–7,505) using the Wilcoxon test for trend. Ordinal logistic regression is a method appropriate for the analysis of CAC data that has a markedly non-normal distribution and a significant proportion of participants with no detectable CAC [26]. CAC scores were divided into ordered outcome categories (0, 1–10, 11–100, 101–400, and >400) using published criteria [27] as described [26,28]. The association of EL mass with CAC was assessed in multivariable models that also included: (1) gender and age (age and age^2); (2) established risk factors, gender, and age; and (3) waist circumference, medications (including hormone replacement therapy in women), established risk factors, gender, and age. Established risk factors included total (or LDL) and HDL cholesterol, triglycerides, systolic blood pressure, smoking (current versus never and ex-smokers), race, exercise (none versus any), fasting glucose, and alcohol intake (drinks per week). The results of ordinal logistic regression are presented as the odds ratio (OR) of being in a higher CAC category comparing the highest quartile of EL mass to the lowest quartile. The proportional odds assumption of ordinal regression was satisfied for all models [29]. Data analysis was performed by the authors (KOB, MR, and MW) using Stata 8.1 (Stata, College Station, Texas, United States).

Results

Characteristics of Study Participants

The characteristics of the study participants are summarized in Table 1. Compared to the 2002 CDC report on the body weight status of US adults [30], there were fewer individuals of healthy weight and more obese individuals in our cohort. The lipid profiles of our participants were similar to those reported for 20- to 59-year-old participants in the National Health and Nutrition Examination Survey III [31].

Table 1. Clinical and Biochemical Characteristics of the Cohort

Characteristic	Men ($n = 466$)	Women ($n = 392$)
	Median (IQR)	Median (IQR)
Age	47 (41–52)	51 (45–57)
Cigarette smokers (%)	10.8	12.9
Blood pressure, systolic	129 (120–136)	125 (111–135)
Blood pressure, diastolic	79 (74–85)	75 (68–82)
BMI (kg/m^2)	27.9 (25.6–30.4)	26.4 (23.1–30.9)
Waist circumference	37.5 (35–41)	32 (29–36)
Total cholesterol (mg/dl)	205 (178–226)	209 (188–238)
LDL-cholesterol (mg/dl)	128 (109–150)	123 (102–146)
HDL-cholesterol (mg/dl)	44 (38–50)	61 (46–70)
Triglycerides (mg/dl)	129 (92–176)	122 (82–156)
ApoA-I (mg/dl)	116 (102–131.5)	145 (125–164)
ApoB (mg/dl)	99 (86–114.5)	98 (82–113)
Pre-heparin EL (ng/ml)	420 (323–565)	472 (314–663)
Post-heparin EL (ng/ml)	1396 (944–2,031)	1165 (730–1,705)

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Distribution of EL Mass Concentrations in Pre-Heparin and Post-Heparin Plasma

Our previous studies indicated that EL, like HL and LPL [32], is an avid heparin-binding protein (unpublished data), and we suspected that it would be released by injection of heparin in humans. Indeed, a preliminary study in 60 individuals with normal lipid profiles indicated an approximately 3- to 4-fold increase in EL mass concentrations from pre-heparin to post-heparin plasma (unpublished data). Interestingly, EL mass was abundant in pre-heparin plasma of the entire SIRCA cohort ($n = 858$), median = 442 (range 324–617) ng/ml. After administration of heparin, median plasma EL concentrations were approximately 3-fold higher than in pre-heparin samples, 1,313 (888–1,927) ng/ml ($n = 510$). There were no significant differences in pre-heparin EL mass concentrations between men and women. While median post-heparin EL concentrations were 16% lower in women, 1,165 (703–1,705) ng/ml, than men, 1,396 (944–2,031) ng/ml, this difference could be attributed to greater weight in men.

The correlation between pre-heparin and post-heparin EL mass was 0.46, $p < 0.001$. Using linear regression, pre-heparin EL mass accounted for 15% of the variation in post-heparin EL mass. While including age and gender added less than 1% each, waist circumference accounted for 5% of the variation in post-heparin EL mass.

Association of EL Mass Concentrations with Nonlipid Cardiovascular Risk Factors

Differences in EL mass between groups in the presence and absence of other risk factors were compared by Kruskal-Wallis χ^2 test. There were significant positive associations between hypertension and both pre-heparin ($p = 0.004$) and post-heparin EL mass ($p = 0.003$). There were significant negative associations between exercise and EL mass in both pre-heparin ($p < 0.001$) and post-heparin ($p = 0.015$) plasma. Smoking was associated with higher post-heparin EL mass ($p = 0.005$).

Correlations of plasma EL mass with age, body mass index (BMI), waist circumference, blood pressure, fasting glucose, and the homeostasis model assessment (HOMA) index, a