ted that EL mass concentrations in pre-heparin plasma were higher than those of LPL [34–37], and injection of heparin resulted in a 3-fold increase in plasma EL mass to concentrations similar to those reported for HL [34,38]. Importantly, within individuals, the pre-heparin and post-heparin EL concentrations are significantly correlated, suggesting that pre-heparin EL mass may be a surrogate marker for total vascular EL expression. Indeed, most of the associations between post-heparin EL mass and metabolic syndrome factors, cardiovascular risk factors, lipids, and CAC were also found for pre-heparin concentrations. This suggests that future studies of the association of EL mass concentrations with cardiovascular outcomes may be able to be performed using routine, pre-heparin plasma samples.

We previously reported that overexpression of EL in hyperlipidemic mouse models reduced concentrations of apoB-containing lipoproteins [39]. However, in this human study, we found that concentrations of EL are positively associated with plasma concentrations of LDL-C, triglycerides, and apoB, but did not correlate with the number of particles in LDL subfractions. This is in contrast to HL, which hydrolyzes remnant lipoproteins and is negatively associated with remnant particles and positively associated with concentrations of small, dense LDL particles [40]. The positive association of EL with apoB-containing lipoproteins is most likely the reflection of the concomitant increase in VLDL and triglycerides [41] and EL mass found in obese individuals and those with metabolic syndrome.

This is the first study to demonstrate that, in humans, EL plasma concentrations are negatively associated with HDL-C concentrations. While the negative correlations found between plasma EL mass concentrations and HDL-C concentrations are decidedly modest, they are highly statistically significant. The correlation between plasma EL mass and HDL-C may, in fact, underestimate the association of EL activity with HDL-C, given that the EL mass assay may only partially reflect the biological activity of EL. This finding is consistent with published reports in mice that overexpression of EL dramatically reduces HDL-C concentrations [6,9], and either inhibition of EL or genetic deletion causes an increase in HDL-C [9,10,11]. Furthermore, we show that EL concentrations are negatively associated with large HDL but positively associated with small HDL, consistent with the model proven in mice [8], whereby EL hydrolyzes surface phospholipids on large HDL, creating smaller, phospholipid-depleted HDL particles. This pattern of negative correlation with large HDL and positive correlation with small HDL has also been reported for HL [42]. Of significance was the lack of correlation between EL mass concentrations and apoA-I. This finding is consistent with the report by Jahangiri [43] that EL remodeling of HDL to smaller particles does not mediate apoA-I dissociation.

The apolipoprotein content of HDL has been shown to influence the activity of HL and EL toward HDL. Several reports by Patsch et al. [44] and Mowri et al. [45] have suggested that an increase in the apoA-II content increases HL hydrolysis of the triglyceride in the larger HDL_2 particle. Recent studies by Hedrick et al. [46], using apoA-II transgenic mice, suggest that apoA-II inhibits HL activity, but the addition of apoA-I partially reverses this inhibition. Boucher et al. [47] found similar results in in vitro studies, in which the addition of apoA-II to apoA-I–containing HDL inhibited substrate hydrolysis by increasing the affinity of HL for the HDL particle. The authors reported that the addition of apoA-II to particles increased HDL size and induced a conformational change in apoA-I. In contrast, Caiazza et al. [48] used spherical HDL of identical size, containing cholesteryl ester as the sole lipid core with phosphatidylcholine and apoA-I, apoA-II, or both to determine the influence of apolipoproteins on EL activity. They found that EL hydrolysis of phospholipids in apoA-II–containing HDL was negligible, measurable in apoA-I–containing HDL, and greatest in spherical HDL containing both apoA-I and apoA-II. While the absence of triglycerides in these spherical particles makes application to native HDL difficult, it does suggest that there are differences in the subpopulations of HDL to which HL and EL bind.

Both Jin et al. [49] and Hirata et al. [50] reported that stimulation of cultured endothelial cells with tumor necrosis factor-α and interleukin 1β caused an increase in EL mRNA expression and protein secretion. Elevated plasma concentrations of tumor necrosis factor-α are found in individuals with metabolic syndrome [51]. The positive correlations between plasma EL mass, obesity, and the other metabolic syndrome factors provide indirect evidence of a cytokine-mediated increase in endothelial EL protein secretion in the setting of obesity and metabolic syndrome. The significant difference in EL mass between lean and obese participants also suggests that this may occur in vivo.

An important finding of this study in healthy asymptomatic individuals is that EL mass concentrations are positively associated with CAC, a measure of subclinical atherosclerosis in humans, even after controlling for cardiovascular risk factors, plasma lipids, and vasoactive medications. The observation that EL tends to be a stronger predictor of CAC in women than in men could reflect the fact that HL activity is lower in women [52]. In the presence of less HL activity, EL might have a greater relative influence on HDL metabolism and atherosclerosis.

Importantly, our analysis was based on measurement of EL mass, not EL activity. LPL and HL activity are generally measured in post-heparin plasma by assaying their triglyceride lipase activity. However, EL has much less triglyceride lipase activity compared with these enzymes. Therefore, EL activity in human plasma will need to be assessed based on its phospholipase activity and will require differentiation of EL-specific activity from other sources of phospholipase activity, such as HL. The development of a validated assay will allow determination of the association between EL mass and activity and EL activity with plasma lipids and atherosclerosis.

In summary, EL mass is present in measurable amounts in pre-heparin plasma, and increases 3-fold after administration of heparin. EL concentrations are inversely associated with total HDL-C concentrations and positively associated with obesity, triglycerides, fasting glucose, and hypertension, factors composing the metabolic syndrome. Finally, plasma EL concentrations are associated with subclinical atherosclerosis independent of all established risk factors, including plasma lipids. These data support the concept that plasma EL concentrations may modulate metabolic dyslipidemia and atherosclerosis. Prospective studies will be needed to determine if EL is, indeed, a risk factor.