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Endothelial lipase plasma levels are increased in both sexes in stable coronary artery disease and only in women with acute coronary syndrome but not associated with the severity of coronary artery disease

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Aim To investigate whether endothelial lipase (EL) plasma levels are increased in stable coronary artery disease (sCAD) and acute coronary syndrome (ACS) patients, as well as to test the association of EL plasma levels and the severity of CAD and sex.

Methods The study was performed as a single-center, cross-sectional, observational research on 72 sCAD and 187 ACS patients in the Sisters of Charity University Hospital Centre, Zagreb, Croatia, between December 1, 2011 and December 1, 2012. EL plasma levels were measured using ELISA.

Results EL plasma levels were significantly higher in sCAD patients (median 311.3 pg/mL, interquartile range [IQR] 250.4-422.6 pg/mL) than in ACS patients (median = 258.7 pg/mL, IQR = 162.1-356.0 pg/mL; $P < 0.001$). EL levels in female ACS patients were significantly higher (median 314.5 pg/mL, IQR 218.3-420.8 pg/mL) than in male ACS patients (median 225.4 pg/mL, IQR 148.7320.1 pg/mL; $P < 0.001$) and similar to the EL levels in the sCAD patients. There was no significant correlation between EL plasma levels and the GENSINI score and between EL plasma levels and the number of atherosclerotic coronary artery segments in either the ACS (rho = -0.09, $P = 0.247$; rho = 0.12, $P = 0.106$, respectively) or sCAD group (rho = 0.04, $P = 0.771$; rho = 0.06, $P = 0.643$, respectively).

Conclusion Our results suggest that EL plasma levels discriminate male but not female patients with different clinical presentations of CAD, as well as female and male ACS patients. EL plasma levels are not significantly correlated with CAD severity.

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Coronary artery diseases (CAD) can cause different clinical presentations compatible with myocardial ischemia/infarction. They can be divided into two main categories: stable CAD (sCAD) and acute coronary syndrome (ACS), the latter ranging from unstable angina pectoris (UAP) to non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI). ACS is almost always associated with erosion or rupture of atherosclerotic plaque, thrombus formation, possible coronary vasospasm, and abrupt reduction of coronary blood flow due to partial or complete occlusion of the infarct-related coronary artery (1). The risk of plaque erosion or rupture is influenced by intrinsic (vulnerability) and extrinsic conditions (local stress due to cyclic stretching, compression, flexion, shear, and pressure fluctuations). Vulnerability of the atherosclerotic plaque is determined by the size of the lipid-rich necrotic core, the thickness of the fibrous cap, and the presence of inflammation (2,3).

Endothelial lipase (EL) is a member of the triglyceride lipase gene family, expressed by vascular endothelial cells (ECs), smooth muscle cells (SMCs), and macrophages (4,5). EL is a phospholipase with a high affinity for high-density lipoprotein-phospholipids (HDL-PL). By acting on HDL, EL alters the structural and functional properties of HDL (6,7), as well as HDL plasma levels in mice (8), rabbits (9), and humans (7,10). EL and bioactive lipids generated by EL-mediated cleavage of HDL-PL (11) were identified as strong inducers of inflammatory cytokines in macrophages (12) and vascular endothelial cells (13).

EL expression can be induced by tumor necrosis factor α, interleukin 1β, and biomechanical forces in vascular endothelial cells (14,15), by angiotensin II and hypertension in vascular SMCs (16), as well as by lipopolysaccharide in macrophages (17).

EL plasma concentrations are strongly associated with C-reactive protein (CRP) and interleukin 6 (IL-6) levels and can be increased by experimentally-induced endotoxemia (18,19). EL is highly expressed in advanced human and mouse atherosclerotic plaques, primarily by macrophages and to a lesser extent by SMCs (20-22). Despite its expression in atherosclerotic lesions and its capacity to augment the secretion of inflammatory cytokines (12,13), neointima formation (23), and monocyte adhesion to the vessel wall (24), as well as its negative impact on HDL plasma levels and functionality (6-8,10), the role of EL in atherogenesis is still obscure.

Studies that have so far investigated the impact of EL on atherosclerosis in mouse models provided controversial results: while EL deficiency in apolipoprotein E-knockout mice attenuated the development of atherosclerotic lesions in one study (25), in an another study no effect of EL deficiency on atherosclerosis development was observed in apolipoprotein E- and low-density lipoprotein (LDL) receptor- knockout mice (26). According to Badelino et al (27), human plasma EL concentrations were significantly associated with subclinical atherosclerosis. They concluded that EL may be a pro-atherogenic factor in humans (27). Another genetic study revealed that the EL gene polymorphism might be associated with ACS (28). However it remained uninvestigated if EL plasma levels are increased in patients with different forms of CAD. Considering the positive association between EL plasma levels and the extent of inflammatory state, together with increased EL expression in atherosclerotic lesions, we hypothesized that EL plasma levels would be increased in sCAD due to persisting low-grade inflammation. Further, we also hypothesized that EL levels were increased by CAD severity (evaluated by the modified GENSINI scoring system and the number of stenotic coronary artery segments) and different in men and women.

MATERIALS AND METHODS

Study design and patients

This study was designed and performed as an observational, cross-sectional clinical study in the Sisters of Charity University Hospital Centre, Zagreb, Croatia. The ACS patients were recruited from the Emergency Department between December 1, 2011 and December 1, 2012. The patients with suspected sCAD scheduled for coronary angiography were recruited from the Department of Cardiology. Written informed consent from each patient was obtained prior to the enrolment to the study according to the Good Clinical Practice and Helsinki Declaration principles (29). The study was approved by the Sisters of Charity UHC and Medical University of Graz Ethics Committees, in accordance with the institutional guidelines.

Inclusion and exclusion criteria

Overall, 226 ACS patients and 111 sCAD patients were screened for enrolment. ACS patients were selected based on either of the typical unstable symptoms, elevation in cardiac markers (creatine kinase, troponine I), dynamic changes in electrocardiogram, and/or

echocardiographic evidence of new loss of viable myocardium or regional wall motion abnormality (30). Coronary angiography was performed in 90% of all suspected sCAD and ACS patients using the radial approach and in the rest with femoral or brachial approach depending on patient individual anatomy. The time interval between arrival to the hospital and coronary angiography was in accordance with the recommendation of the European Society of Cardiology (within 2 hours for STEMI patients, 2-72 hours for other ACS patients, indefinite for sCAD patients) (31-33).

Exclusion criteria were a patient's refusal to participate in the study and/or to undergo coronary angiography, allergy to intravenous contrast, acute aortic dissection, chronic heart failure with ejection fraction (EF) below 40%, chronic renal failure with serum creatinine ≥200 mmol/L, a chronic autoimmune and/or infectious disease, and decompensated liver cirrhosis (Child-Pugh class B and C).

Patient characteristics, history, and medication

The patients' demographic data were recorded by the investigator in the data collection form. Special consideration was given to patients' symptoms in order to determine the CAD type. Previous antihypertensive, statin, and antiplatelet treatments and the presence of major atherosclerotic risk factors were noted. Heart rhythm was determined and blood pressure calculated as the average of three measurements. Hypertension was diagnosed according to the criteria of the European Society of Cardiology (34). Hypertension was defined as systolic blood pressure (SBP)≥140 mm Hg and/or diastolic blood pressure (DBP)≥90 mm Hg, and patients' history of using antihypertensive medications was also considered. Patients with diabetes were considered as those with dietary treatment, antidiabetic medication, or current fasting plasma glucose levels higher than 7.0 mmol/L (35). Hyperlipidemia was defined as a low-density lipoprotein (LDL)-cholesterol level higher than 3.5 mmol/L, or taking a lipid-lowering drug. Patients were classified as current smokers (smoking more than five cigarettes per day within the past 3 months) or non-smokers.

Coronary angiography

Angiographic films were reviewed visually by two independent interventional cardiologists blinded to the patients' clinical information. The mean of two measurements was calculated for each patient. The CAD severity was evaluated using 1) the number of stenotic coronary artery segments (36) and 2) the modified GENSINI scoring system (37).

The modified GENSINI scores were derived as described previously (38). Briefly, the number of lesions, their severity, and their respective locations were evaluated. The coronary vasculature was divided into 27 segments, and each segment was graded from 0.5 to 5.0, reflecting the importance of location, ie, yielding the location score. The severity was scored as follows: <25%=2; 26% to 50%=4; 51% to 75%=8; 76% to 90%=16; 91% to 99%=32; total occlusion=64. The product of the severity score and location score constitutes the score for any given segment, and their sum makes up the score for a given subject.

Laboratory assays

Blood samples were collected immediately at hospital arrival for ACS patients. For sCAD patients, blood was collected in the morning following hospitalization after overnight fasting. The average time from the onset of symptoms (pain) to arrival to hospital for ACS patients with ST elevation myocardial infarction was 215 minutes. For other ACS patients (NSTEMI and UAP) the time was longer (hours to days) and sometimes not easy to determine because of the nature of the clinical presentation (repetitive pain).

After blood sampling, laboratory measurements were immediately performed and plasma samples were frozen at -30°C for further EL and IL-6 analysis. The routine laboratory measurements were performed on an automated, multichannel selective analyzer Modular (Roche Diagnostics, Mannheim, Germany). IL-6 concentrations were measured using a specific chemiluminescent ELISA (QuantiGlo; R&D Systems, Wiesbaden-Nordenstadt, Germany) and EL protein levels by Human Endothelial Lipase Assay Kit (TaKaRa, Takara Bio Europe S.A.S., Saint-Germain-en-Laye, France), according to the manufacturer's instructions.

Statistical analysis

Normality of data distribution was assessed using Smirnov-Kolmogorov test, and appropriate nonparametric tests were used. Differences in categorical values between the ACS and sCAD groups were analyzed using χ^2 test. Differences in quantitative values between the ACS and sCAD were analyzed using Kruskal-Wallis test, and for individual group differences using Mann-Whitney U test and Bonferroni correction where appropriate. Spearman's Rho nonparametric test was used to measure the strength of cor-

relation between two variables. Ordinary least squares (OLS) regression was used to determine the predictors of EL plasma levels. The OLS regression model was statistically significant ($P < 0.001$) with 15.6% of explained EL variance (r^2). *P* values below 0.05 were considered significant except for multiple comparisons where the significance was dependent on the number of simultaneously tested hypotheses. The data analysis software system IBM SPSS Statistics, version 21.0 (IBM, Armonk, NY, USA), was used in the statistical analyses.

RESULTS

Sample size and clinical characteristics

Out of 226 ACS patients and 111 sCAD patients assessed for eligibility, 39 patients from each group were excluded due for the presence of exclusion criteria (Figure 1). The final study population included 187 ACS and 72 sCAD patients.

sCAD and ACS groups did not differ significantly in age, sex, and body mass index (BMI) (Table 1). In the ACS group there were significantly more smokers, whereas in the sCAD group there were more diabetics and patients with present or treated hyperlipoproteinemia (Table 1). The mean arterial pressure (MAP) and left ventricle (LV) EF were significantly lower in ACS than in sCAD group (Table 1). ACS and sCAD groups did not differ in the number of segments, whereas the GENSINI score was significantly higher in the ACS group ($P < 0.001$) (Table 1). The 30-day mortality rate was higher in the ACS than in the sCAD group (4.8 vs 0%), but not significantly ($P = 0.054$). There were more men in both the ACS and sCAD group (Supplementary Table 1). In the ACS group significantly more men than wom-

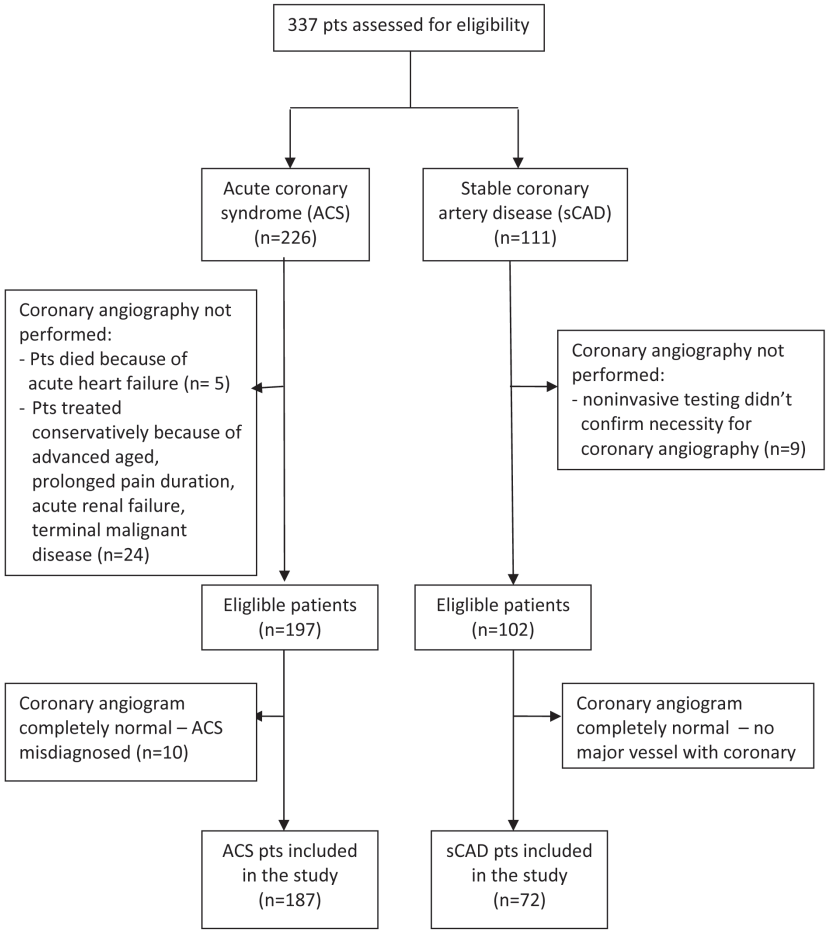

FIGURE 1. Flowchart of the study. Patients (Pts).

en were smokers and significantly more women than men were treated with ASA (Supplementary Table 1). In the sCAD group MAP was significantly higher in women than in men (Supplementary Table 1).

Laboratory parameters

Glucose levels, total cholesterol levels, and LDL-cholesterol levels were significantly higher in ACS than in sCAD group ($P < 0.001$) (Table 2). HDL-cholesterol and triglyceride levels were similar in the sCAD and ACS patients (Table 2). There was no significant difference in high-sensitive C-reactive protein (hsCRP) levels between the ACS and sCAD patients, whereas IL-6 levels were significantly higher in ACS patients (Table 2).

Importantly, EL plasma levels were significantly higher in sCAD than in ACS patients-(Table 2). This finding, namely normal EL levels in ACS patients, prompted us to investigate the predictors of EL levels in sCAD and ACS.

Predictors of EL plasma levels in sCAD and ACS patients

The OLS regression model, which included both ACS and sCAD patients, identified that belonging to the ACS group was a negative predictor of EL plasma levels, whereas both

TABLE 1. Clinical characteristics and medications*

	ACS (n = 187)	sCAD (n = 72)
Age (years); median (IQR)	65.0 (55.0-76.0)	64.0 (56.0-71.0)
Sex; n (%)		
men	129 (69.0)	46 (63.9)
women	58 (31.0)	26 (36.1)
Diabetes mellitus; n (%)†	27 (14.4)	22 (30.6)
Hyperlipoproteinemia; n (%)‡	50 (26.7)	33 (45.8)
Smoking cigarettes; n (%)§	68 (36.4)	16 (22.2)
MAP (mmHg); median (IQR)‖	90.0 (80.8-96.7)	96.7 (92.1-106.7)
BMI (kg/m^2); median (IQR)	28.0 (25.1-31.0)	27.2 (25.1-30.1)
LV EF (%); median (IQR)¶	50.0 (46.5-55.0)	60.0 (54.8-60.0)
Number of segments; median (IQR)	2.0 (1.0-4.0)	2.0 (2.0-4.0)
GENSINI score; median (IQR)**	48.0 (32.0-80.0)	27.0 (10.3-39.5)
ASA before hospitalization; n (%)	40 (22.0)	48 (66.6)
Statins before hospitalization; n (%)	34 (18.7)	39 (54.1)

*IQR – interquartile range; MAP – mean arterial pressure; BMI – body mass index; LV EF – left ventricular ejection fraction; ACS – acute coronary syndrome; sCAD – stable coronary artery disease; ASA – acetyl salicylic acid.
†χ^2, $P = 0.003$.
‡χ^2, $P = 0.003$.
§χ^2, $P = 0.029$.
‖P < 0.001 Mann-Whitney U test.
¶P < 0.001 Mann-Whitney U test.
**P < 0.001 Mann-Whitney U test.

TABLE 2. Laboratory results*

Group	ACS (n = 187) M (IQR)	sCAD (n = 72) M (IQR)	*P* (Mann-Whitney U test)
Total cholesterol (mmol/L)	5.3 (4.4-6.2)	4.8 (3.7-5.5)	<0.001
LDL-cholesterol (mmol/L)	3.5 (2.8-4.2)	2.8 (2.0-3.8)	<0.001
HDL-cholesterol (mmol/L)	1.1 (0.9-1.3)	1.1 (0.9-1.2)	0.909
Glucose (mmol/L)	7.8 (6.5-10.2)	5.9 (5.1-7.7)	<0.001
Triglycerides (mmol/L)	1.4 (1.0-2.1)	1.5 (1.1-2.2)	0.233
hsCRP (μg/mL)	3.7 (1.6-7.1)	2.3 (1.4-5.1)	0.057
IL-6 (pg/mL)	6.3 (4.3-14.2)	3.0 (2.0-5.6)	0.001
EL (pg/mL)	258.7 (162.1-356.0)	311.3 (250.4-422.6)	<0.001

*ACS – acute coronary syndrome; sCAD – stable coronary artery disease; M – median; IQR – interquartile range; hsCRP – high sensitivity C-reactive protein; IL-6 – interleukin-6; LDL – low-density lipoprotein; HDL – high-density lipoprotein; EL – endothelial lipase.

hsCRP and female sex were positive predictors of EL plasma levels (Table 3).

Sex markedly impacts the EL and hsCRP plasma levels in ACS patients, but not in sCAD patients

To examine how sex impacts EL plasma levels, we compared EL levels in female and male sCAD and ACS patients. We found a evident sex difference in the ACS group, namely, significantly higher EL levels in female ACS patients than in male ACS patients ($P < 0.001$) (Figure 2A). In sCAD patients EL levels were not significantly different between women and men (Figure 2A). EL levels in female ACS patients were similar to the levels in female sCAD patients, whereas the levels in male ACS patients were significantly lower than in male sCAD patients (Figure 2A). Similarly as for EL, hsCRP plasma levels were also significantly higher in female than in male ACS patients ($P < 0.001$), with no sex differences in sCAD patients ($P = 0.841$) (Figure 2B). IL-6 plasma levels were higher in female ACS patients than in male ACS patients, however, without reaching statistical significance (Figure 2C). Glucose plasma levels and plasma lipids except HDL-cholesterol, which was significantly higher in ACS women than in men (men: median 1.1 mmol/L,

TABLE 3. Predictors of endothelial lipase plasma levels*

	Unstandardized coefficients		Standardized coefficients		95% confidence Interval for B		
	B	standard error	beta	t	lower bound	upper bound	*P*
(Constant)	295.08	113.20		2.61	72.02	518.14	0.010
ACS group	-104.23	29.49	-0.25	-3.53	-162.32	-46.14	<0.001
Age (years)	1.71	1.07	0.10	1.59	-0.40	3.82	0.112
Female sex	63.58	27.02	0.16	2.35	10.36	116.81	0.019
hsCRP (mg/L)	1.16	0.40	0.18	2.92	0.38	1.93	0.004
Total cholesterol (mmol/L)	-15.40	22.58	-0.11	-0.68	-59.87	29.07	0.496
HDL-cholesterol (mmol/L)	-33.59	34.40	-0.07	-0.98	-101.35	34.16	0.330
LDL-cholesterol (mmol/L)	7.78	22.04	0.05	0.35	-35.64	51.21	0.724
Triglycerides (mmol/L)	7.19	13.27	0.04	0.54	-18.96	33.33	0.589
Glucose (mmol/L)	4.96	2.95	0.11	1.68	-0.86	10.78	0.094
MAP (mmHg)	-0.78	0.87	-0.06	-0.90	-2.50	0.93	0.370

*ACS – acute coronary syndrome; sCAD – stable coronary artery disease; hsCRP – high sensitive C-reactive protein; HDL – high density lipoprotein; LDL – low density lipoprotein; MAP – mean arterial pressure

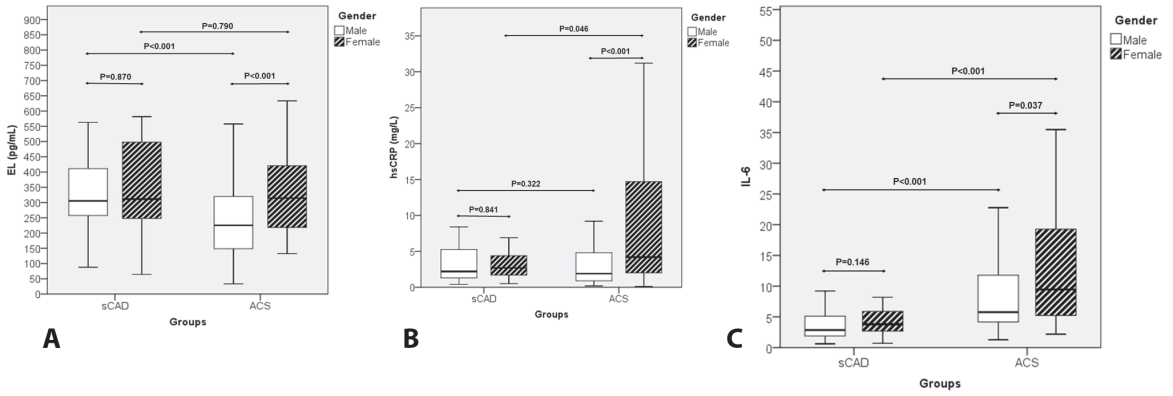

FIGURE 2. Endothelial lipase (EL), high sensitivity C-reactive protein (hsCRP), and interleukin-6 (IL-6) levels in the study groups: (**A**) EL and (**B**) hsCRP protein levels were measured in the serum of 46 stable coronary artery disease (sCAD) men, 26 sCAD women, 129 ACS men, 58 acute coronary syndrome (ACS) women, by ELISA. $P \leq 0.0083$ was considered statistically significant. (**C**) IL-6 protein levels were measured in the serum of 46 sCAD men, 26 sCAD women, 129 ACS men, and 58 ACS women by ELISA. $P \leq 0.0125$ was considered statistically significant. The boundaries of the box indicate the lower and upper quartiles, the horizontal line the median and the error bars the 95% confidence interval.

IQR 0.9-1.3 mmol/L; women: median: 1.2 mmol/L, IQR 1.0-1.4 mmol/L, $P=0.007$), were similar in men and women in both groups (not shown).

Relationship between EL levels and inflammatory markers

We found no correlation between EL levels and hsCRP in either sCAD or ACS patients. However, when the correlations were examined in women and men separately, a strong significant correlation between EL and hsCRP levels was observed only in female ACS patients (rho=0.361, $P=0.005$). There was no correlation between EL and IL-6 plasma levels (not shown).

Relationship between EL levels and CAD severity

We found no correlation between EL plasma levels and the GENSINI score, and no correlation between EL plasma levels and the number of atherosclerotic coronary artery segments in either the ACS (rho=-0.09, $P=0.247$; rho=0.12, $P=0.106$, respectively) or sCAD group (rho=0.04, $P=0.771$; rho=0.06, $P=0.643$, respectively).

DISCUSSION

The main finding of this study is that EL plasma levels were increased in both sexes in sCAD and were not correlated with CAD severity evaluated by the modified GENSINI scoring system and the number of stenotic coronary artery segments. However, EL plasma levels were increased only in women with ACS.

Given the role of HDL in the pathogenesis of atherosclerosis (39-41), together with the established impact of EL on HDL metabolism (7,8) and the positive relationship between EL and inflammation (18), EL is considered to be a proatherogenic molecule. In our previous work we demonstrated a higher EL protein expression in symptomatic and unstable carotid plaques, compared with the plaques found in patients without neurological symptoms and with a stable plaque phenotype (22). Furthermore, in patients with significant carotid artery stenosis, EL plasma levels were higher in the symptomatic than in the asymptomatic group (42).

Considering the positive relationship between EL plasma levels and the extent of inflammatory state (18,19), as well as coronary (27) and carotid atherosclerosis (22), we hypothesized that EL plasma levels were different in different clinical presentations of CAD and were related to CAD severity. We hypothesized that EL plasma levels would be increased in sCAD due to persisting low-grade inflammatory state. Although the relative contribution of coronary compared to systemic endothelium in terms of EL production is rather small, it is possible that compromised endothelial function in acute compared to chronic forms of CAD was at least in part responsible for decreased EL levels in ACS. The initial data analyses revealed significantly higher EL levels in the sCAD group, which also had significantly lower IL-6 levels compared to the ACS group. This was contradictory to previous studies showing the positive relationship between EL and acute inflammatory markers, such as IL-6 and hs-CRP (18,19).

The most striking and interesting finding of the present study emerged upon addressing the role of sex in the determination of EL levels in sCAD and ACS patients. We found increased EL levels in female ACS patients, similar to the levels in sCAD patients, but markedly lower EL levels in male ACS patients. This observation, together with the higher prevalence of men (69%) having low EL levels in the ACS group. explains the low EL levels in that group. To our knowledge this is the first report on a sex-related difference in EL plasma levels in ACS patients. Besides EL, hsCRP plasma levels were also higher in female ACS patients than in male ACS patients. Although hormone replacement therapy might at least in part explain the increased hsCRP levels (43) in women with ACS, the responsible underlying mechanism remained unexplained (44,45). Our results add a new piece of evidence to the long list of sex differences in CAD patients (44,46). It remains to be determined whether EL plasma concentration might be a useful biomarker for risk stratification and/or choice of therapeutic approaches for patients with ACS. We speculate this might be due to prolonged endothelial damage of small coronary vessels in women.

Interestingly, although EL plasma levels correlated with hsCRP levels in previous studies (19,47), we found a significant correlation only in the female ACS patients with increased both EL and hsCRP. It is not clear why correlations were not found in other groups. One can speculate that the simultaneous response of both EL and hsCRP to acute inflammatory stimuli, as in female ACS patients, is required to observe a relationship. Furthermore, the lack of association between EL and inflammatory markers in the present study may be due to the disruption of the association by medication (48). In contrast to previous studies where EL levels and inflammatory markers were determined in the

plasma (19,47), we measured them in the serum. In light of previous findings showing different hsCRP levels in plasma and serum prepared from the same blood (49), it remains to be established whether the experimental procedure disrupts the association between EL and inflammatory marker levels.

In the present study EL levels did not correlate significantly with HDL-C levels either in sCAD or ACS patients. The lack of association between EL and HDL-C levels might be partially explained by the fact that high levels of EL mass, as measured by ELISA, do not necessarily have to be accompanied by increased plasma EL activity, considering the reduced phospholipase activity of various genetic variants (7,10).

More importantly, the mass of cholesterol that is carried by the HDL particles, ie, HDL-cholesterol, is not a sensitive parameter of the atheroprotective properties of HDL. Rather, the direct measurement of HDL function (50) or the assessment of HDL particle concentrations is a much better estimation of the cardioprotective properties of HDL. Indeed, HDL functionality, but not HDL-cholesterol levels, has been demonstrated to predict long-term mortality (51). Of note, EL was found to reduce the functionality of HDL (7). Therefore, the lack of association between EL and HDL-cholesterol levels has no significance, because the important parameter is the EL-induced alteration of HDL functionality.

In the present study we found no significant correlation between EL plasma levels and CAD severity, evaluated by the GENSINI score and by quantifying the number of diseased segments of the coronary artery. In patients with significant carotid stenosis, we previously found that the intensity of the plaque EL immunostaining was related to plaque vulnerability but not associated with EL plasma levels (22,42). Accordingly, it is likely that in coronary artery plaques of sCAD and ACS patients, like in carotid plaques, EL expression levels in coronary atherosclerotic lesions are related to CAD severity, but that the augmented local EL expression does not contribute significantly to the overall EL abundance in plasma. Therefore, in the present study the dissociation between local and systemic EL levels might be responsible for the lack of association between EL levels and CAD severity. Considering the association of EL plasma levels with atherogenic lipid profile (27), together with improved functionality of HDL in individuals with genetically decreased EL plasma levels (52,53), EL should be considered a potential therapeutic target in dyslipidemia. However, in this study HDL-C and triglycerides levels did not differ between sCAD and ACS patients.

Both diabetes mellitus type 2 and hyperlipoproteinemia, which had higher prevalence in the sCAD patients, might have contributed to higher EL levels in this group due to their association with low-grade chronic inflammation. Furthermore, both ASA and statins, more frequently used before hospitalization in sCAD patients, might have additionally impacted EL levels (54).

The main study strength is its comprehensive design as a single-center observational and non-interventional study, with a highly structured protocol and predefined statistical analyses. However, the main limitation of this study was the sample size of the sCAD group which was modest and therefore our ability to definitely evaluate the association between EL plasma levels and CAD severity in the sCAD group was limited.

Based on our results we conclude that EL plasma levels discriminate male patients with different clinical presentations of CAD, ie, sCAD and ACS, as well as female and male ACS patients. EL plasma levels are not related to CAD severity. It remains to be determined whether EL might provide prognostic information on the development of cardiovascular events or predict the outcome in patients with ACS, particularly in women, in whom the diagnosis and risk assessment of CAD is reportedly more difficult than in men.

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Declaration of authorship All authors have substantially contributed to the conception and design of the work, the acquisition, analysis and interpretation of data for the article. All authors contributed to the drafting of the work or revising it critically for important intellectual content and final approval of the version.

Competing interests All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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