Serum CETP and PLTP activity in middle-aged men living in urban or rural area of the Lower Silesia region. PURE Poland sub-study

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Submitted: 11 June 2015
Accepted: 9 September 2015

Arch Med Sci 2016; 12, 4: 704–714
DOI: 10.5114/aoms.2016.60950
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Abstract

Introduction: The dependence of lipid transfer proteins on significant pro-atherogenic factors is unclear. The aim of the study was to evaluate serum cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) activity in relation to lipid disturbances in men living in an urban or rural area.

Material and methods: A group of 427 men, volunteers for the Prospective Urban Rural Epidemiology (PURE) sub-study – 263 urban inhabitants (aged 51.9 ±6.0) and 164 residents of villages (aged 51.1 ±5.9) – were examined. In the multivariable linear regression model, the following factors were included as potential confounders: age, body mass index (BMI), smoking, alcohol consumption, hs-C-reactive protein reaction (hs-CRP) and co-existence of chronic diseases.

Results: In multiple linear regression models, site of residence (urban or rural area) was the most important independent and consistent predictor of CETP and PLTP activity; β coefficients (95% CI) for CETP (0.18) and PLTP (–0.29) were significant at levels of $p < 0.001$. Three-way analysis of variance showed no effect of smoking or moderate alcohol consumption on lipid transfer proteins; however, CETP activity showed an interaction effect between these risk factors. In the group of all men, CETP activity was significantly and positively correlated with total cholesterol ($r = 0.24$), low-density lipoprotein cholesterol ($r = 0.18$), and non-high density lipoprotein cholesterol ($r = 0.21$), whereas PLTP activity was correlated with BMI ($r = 0.12$). Body mass index in rural men was higher than in the urban male population.

Conclusions: Increased PLTP activity, recognized as a pro-atherogenic factor, and decreased CETP activity, known as a protective factor, both observed in men living in rural areas, are probably conditioned by nutritional and/or genetic factors.

Key words: cholesteryl ester transfer protein, phospholipid transfer protein, men.

Introduction

The atherogenic role of plasma lipid transfer proteins, such as cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP), in humans is still not fully explained. It is known that these circulating lipid transfer glycoproteins bind and transfer a number of amphi-

pathic compounds and determine the cholesterol content in the intima of the arterial vessel wall. The overload of cholesterol in the arterial wall is one of the main causes of arteriosclerosis. However, the effect of plasma lipid transfer proteins on cholesterol accumulation in the vessel wall, reverse cholesterol transport and cholesterol's further changes remains unclear [1, 2].

The cholesteryl ester transfer protein promotes the exchange of triglycerides from very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) particles for cholesterol ester in high-density lipoprotein (HDL) particles [3, 4]. In this way, CETP enhances triglyceride content in HDL, and redistributes and remodels HDL into small HDL_3 particles, which are subsequently hydrolyzed by lipase [4, 5]. Increased CETP activity may be a major determinant of low HDL cholesterol [6]. Animals naturally depleted in CETP (e.g. mice and rats) have high HDL-C and low LDL-C plasma levels, whereas rabbits, having a naturally high CETP activity with high LDL and low HDL concentrations, are sensitive to developing experimental atherosclerosis [7, 8]. Since for many years CETP has been considered an atherogenic factor, clinical studies using a potent CETP inhibitor, such as torcetrapib, were performed in patients at high risk for coronary events. However, the trial with torcetrapib was terminated prematurely because of an increased risk of death and cardiac events in patients receiving this drug. Furthermore, torcetrapib is thought to have off-target effects on blood pressure [9]. Dalcetrapib development was interrupted by Roche due to neutral results of the DAL-OUTCOMES study. At present, there are two large phase 3 trials studying the effect of CETP inhibition on clinical endpoints: REVEAL (anacetrapib) and ACCELERATE (evacetrapib). In patients already receiving statin therapy, these inhibitors cause plasma HDL to increase and LDL to decrease [6, 10]. The final data of prospective clinical trials are expected in 2017.

The plasma phospholipid transfer protein also plays an essential role in the metabolism of HDL [11, 12]. The role of PLTP includes transfer of surface remnants from triglyceride-rich particles, VLDLs, and chylomicrons to HDL particles, maintenance of HDL levels, and modulation of the size and composition of HDL [2, 13]. In experiments carried out on mice, PLTP overexpression induces atherosclerosis, whereas PLTP deficiency reduces it [14]. The coexistence between high PLTP and low HDL concentrations was also found in humans [2]. Additionally, the pro-atherogenic effect of elevated PLTP activity is due to an increase in production of apolipoprotein B, decrease in anti-oxidative potential and a positive correlation between PLTP and inflammatory factors, i.e. hs-CRP [15, 16]. In clinical studies, high levels of PLTP were observed in patients diagnosed with obesity, type 2 diabetes mellitus (T2DM) or hypertriglyceridemia [16, 17], as well as in patients with coronary heart disease (CHD) [15]. Recently, PLTP has been determined as an independent risk factor for human coronary heart disease [14, 18].

It follows that the biological roles of CETP and PLTP must be carefully monitored and all conditions determining the mass/activity of these proteins should be specified. Previous studies showed that in the middle-aged population of the Lower Silesia region in Poland, the place of residence (urban/rural area) had a significant impact on the lipid pattern. In women, this pattern was more atherogenic in residents of villages than residents of urban areas [19]. Moreover, the global cardiovascular risk was higher in men than in women [20].

The aim of this study was to evaluate serum CETP and PLTP activity in middle-aged men living in urban and rural regions of Lower Silesia, in relation to atherogenic changes in lipid pattern. A comparative analysis of PLTP and CETP activity in men diagnosed with hypercholesterolemia or atherogenic (residual) dyslipidemia vs. normolipidemic men was performed. The activity of CETP and PLTP, depending on the place of residence, smoking habits, alcohol consumption, and presence of chronic diseases, was estimated. In the next step, we plan to specify the activity of CETP and PLTP, and evaluate the impact of the most common hormonal disorders on lipid transfer proteins in women.

Material and methods

Study participants and design

Four hundred and twenty-seven middle aged men were recruited from communities in and around Wroclaw, Lower Silesia region in Poland, from January 2009 to June 2010. The research was conducted as part of the Polish sub-study of the Prospective Urban Rural Epidemiology (PURE) study. All studied subjects were of Polish origin and volunteers. All participants provided informed consent. The baseline clinical visits included measurement of anthropometric data, taking laboratory tests in a fasting state, questionnaires and interviews. Body weight, height, waist and hip circumferences were measured using calibrated equipment and standardized methodology. Body mass index (BMI) was estimated as the ratio of weight to height squared (kg/m^2). Waist-to-hip ratio (WHR) was calculated. Alcohol use was measured as alcoholic drinks consumed per month. Smoking burden was evaluated by current cigarette smoking (cigarettes/day) and pack-years (current cigarettes/day × years of smoking). The patient selection criteria for this study were: sex

(male), age (middle), place of residence (urban or rural area), general health (good) and differential lipid pattern (from normal to dyslipidemia). It was planned to select equal groups of inhabitants of towns and villages, similar for baseline lipid pattern. However, the villagers reported to be less keen on participating in the research, so the group of rural men was smaller. The studied population was divided into: a hypercholesterolemic group (the group of men with fasting total cholesterol ≥ 5.0 mmol/l and/or LDL-C ≥ 3.0 mmol/l), a residual dyslipidemic group (men with triglycerides ≥ 1.7 mmol/l and HDL-C < 1.0 mmol/l) and a normolipidemic group (men without lipid abnormalities). The criteria used for this division were consistent with the guidelines of the Polish Forum for Prevention of Cardiovascular Diseases, updated in 2012 (http://www.pfp.edu.pl). The study was approved by the Polish Ethics Committee: no. KB-443/2006.

Biochemical measurements

Venous blood was taken from subjects after 12 h of fasting, and centrifuged at 1000 g for 20 min at 4°C. Each serum sample was divided and put into 3 tubes and stored at a temperature of –80°C. Serum lipids were measured using standard methods. Total serum cholesterol (TC), triglycerides (TG) and HDL-C were measured using the SPINREACT (Sant Esteve De Bas, Girona, Spain) enzymatic assay. LDL-C was estimated, among patients with a TG concentration lower than 4.52 mmol/l, as the result of TC – HDL-C – TG/5 (Friedewald formula). The QUANTOLIP HDL (Technoclone GmbH, Vienna, Austria) precipitation test was used to measure HDL_2 and HDL_3 cholesterol. The serum non-HDL cholesterol was calculated as the difference between total and HDL cholesterol concentrations. The serum high-sensitivity C-reactive protein (hs-CRP) was determined using the CardioPhases CRP Dade Behringer preparation with the molecular immune-nephelometric method, according to N Rheumatology Standard SL (BCR-CRM 470).

Serum CETP and PLTP activity assays

Serum CETP and PLTP activities were determined using the CETP Activity Assay Kit, and PLTP Activity Assay Kit (BioVision Research Products, 2455-D Old Middlefield Way, Mountain View, CA 94043 USA) with the fluorescence spectrophotometer HITACHI F-2500. The CETP assay uses a synthetic fluorescent CE donor particle and apo-B containing lipoprotein acceptor particles. CETP-mediated transfer was determined by an increase in fluorescent intensity in the acceptor. The serum PLTP assay uses a fluorescent phospholipid donor and a synthetic acceptor, and, again, PLTP-mediated transfer was measured by an increase in fluorescent intensity. Intra- and inter-assay coefficients of variation for both assays ranged from 11% to 15%, similarly as in fluorometric assay procedures that are described by others [21]. Previously, Chen *et al.*, in order to validate the fluorometric assay, compared results with those obtained by the classic radiolabeled method and confirmed the high degree of correlation between the two methods [22].

Statistical analysis

Results are presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Depending on the type of variable distribution, parametric or non-parametric methods of analysis were used. In case of normal distribution, *t*-tests were applied and the statistical significance between means was calculated using the ANOVA test. In the case of qualitative variables, non-parametric tests were used. The association between place of residence and lipid metabolism was investigated. The independent variable was defined as urban or rural area, and was individually analyzed in relation to protein and lipid parameters in the multivariable linear regression model. The following variables were included as potential confounders in the analyses: age, BMI (BMI ≤ 25 kg/m^2 was treated as normal, > 25 kg/m^2 as overweight, > 30 kg/m^2 as obesity), tobacco use (smokers or non-smokers), alcohol consumption (moderate drinkers or non-drinkers), hs-CRP (≤ 5 mg/l was treated as normal, > 5 mg/l as increased) and co-existence of coronary heart disease or diabetes mellitus (present or absent). Three-way analysis of variance (using place of residence, smoking habits and alcohol consumption as independent factors) was also applied with one- or multi-dimensional significance tests. Correlations between variables were evaluated using Spearman's correlation coefficient. A *p*-value less than 0.05 was accepted as statistically significant. All analyses were conducted using the STAT statistical package version 12.0 (Statistica 12 PL. StatSoft).

Results

The study included 263 urban inhabitants aged 51.9 ±6.0 years and 164 rural inhabitants aged 51.1 ±5.9 years. In this population, 123 (28.8%) men were tobacco smokers and 129 (30.2%) men reported moderate alcohol consumption (1 to 4 alcohol units per day). The percentage of smokers and alcohol drinkers was slightly (not significantly) higher among rural residents compared to urban residents. In groups of smokers or moderate drinkers, inhabitants of cities were older by about

one year in comparison to rural area inhabitants. The BMI was higher ($p < 0.05$) in rural inhabitants than in urban inhabitants. The number of men diagnosed with a chronic disease, such as T2DM or CHD, expressed as a percentage of all subjects, was similar in both groups. Simultaneously, except for hs-CRP, which was higher in men from rural areas than in men from cities ($p < 0.01$), other biochemical findings (lipid pattern) did not differ significantly between these two groups (Table I).

In the total population of 427 men, linear correlations between total and LDL cholesterol ($r = 0.885$; $p < 0.001$), total and non-HDL cholesterol ($r = 0.944$; $p < 0.001$), and between TG and HDL_3 cholesterol ($r = –0.375$; $p < 0.001$), were observed. There were also statistically significant relationships, although with lower coefficients, between CETP activity and total cholesterol ($r = 0.238$; $p < 0.001$) (Figure 1), CETP activity and LDL-C ($r = 0.179$; $p < 0.01$) (Figure 2), and between CETP activity and non-HDL-C ($r = 0.212$; $p < 0.001$). The PLTP activity was significantly and positively correlated with BMI ($r = 0.123$; $p < 0.01$).

In comparison to urban men, serum CETP activity in rural men was lower ($p < 0.001$), whereas PLTP activity was higher ($p < 0.001$) (Table II). Multiple linear regression analyses adjusted for age, BMI, smoking habits, alcohol consumption and presence of T2DM or CHD, showed an association between place of residence (urban or rural area) and activity of lipid transfer proteins.

Table I. Characteristics of studied groups. Urban men to rural men group comparison

Parameter	Urban men ($n = 263$)	Rural men ($n = 164$)
Age, mean ± SD [years]	51.9 ±6.0	51.1 ±5.9
BMI, median (IQR) [kg/m^2]	27.7 (25.4–30.5)	28.4 (26.1–31.5)*
WHR, mean ± SD	0.95 ±0.07	0.96 ±0.06
Smokers, *n* (%)	60 (23.1)	63 (38.6)
Age [years]	52.4 ±5.9	50.9 ±6.2
Moderate drinkers, *n* (%)	74 (28.5)	55 (33.7)
Age [years]	51.9 ±6.0	50.8 ±6.1
T2DM, *n* (%)	17 (6.6)	6 (3.7)
Age [years]	49.3 ±7.0	51.6 ±6.3
CHD, *n* (%)	16 (6.2)	5 (3.1)
Age [years]	49.5 ±6.5	54.4 ±5.9
Lipid-lowering treatment, *n* (%)	11 (4.2)	1 (0.6)
Total C, mean ± SD [mmol/l]	5.06 ±0.99	5.27 ±1.27
LDL-C, mean ± SD [mmol/l]	2.99 ±0.89	3.08 ±1.10
HDL-C, mean ± SD [mmol/l]	1.33 ±0.30	1.38 ±0.45
HDL_2-C, mean ± SD [mmol/l]	0.34 ±0.14	0.35 ±0.19
HDL_3-C, mean ± SD [mmol/l]	0.99 ±0.22	1.03 ±0.29
Non-HDL-C, mean ± SD [mmol/l]	3.73 ±1.01	3.90 ±1.27
TG, mean ± SD [mmol/l]	1.65 ±1.09	1.81 ±1.33
hs-CRP, median (IQR) [mg/l]	0.88 (0.51–1.86)	1.22 (0.68–2.27)**

,Statistically significant differences in comparison to urban men; *p < 0.05; **p < 0.01. WHR – waist-to-hip ratio, C – cholesterol, TG – triglycerides, hs-CRP – high-sensitivity C-reactive protein.*

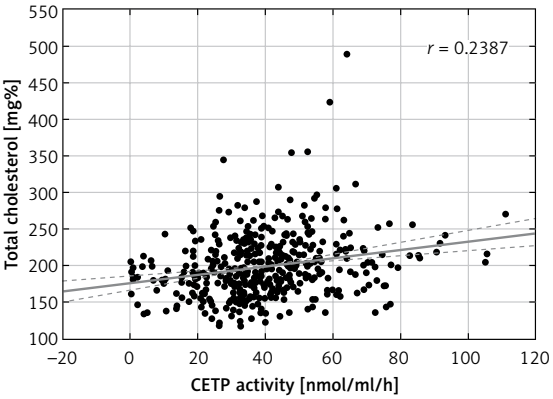


Figure 1. Correlation between CETP activity and total cholesterol level in the group of all men ($p < 0.0001$)

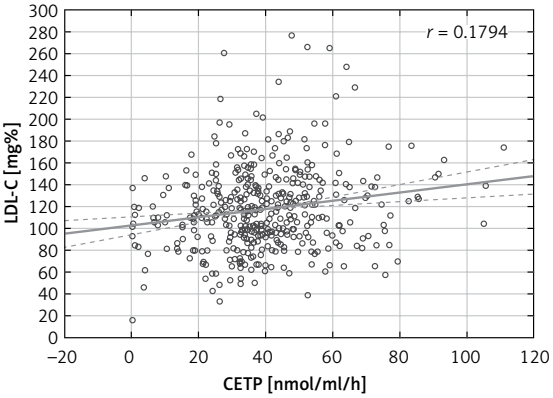


Figure 2. Correlation between CETP activity and LDL cholesterol level in the group of all men ($p < 0.01$)

Table II. Lipid transfer protein activity in urban and rural men

Parameter	Urban area ($n = 263$)	Rural area ($n = 164$)
CETP [nmol/ml/h]	42.7 ±17.7	34.6 ±17.4***
PLTP [nmol/ml/h]	63.8 ±15.5	75.3 ±17.1***

****Statistically significant difference between groups of urban and rural men; p < 0.001.*

Table III. Association between site of residence and lipid transfer protein activity in middle-aged men

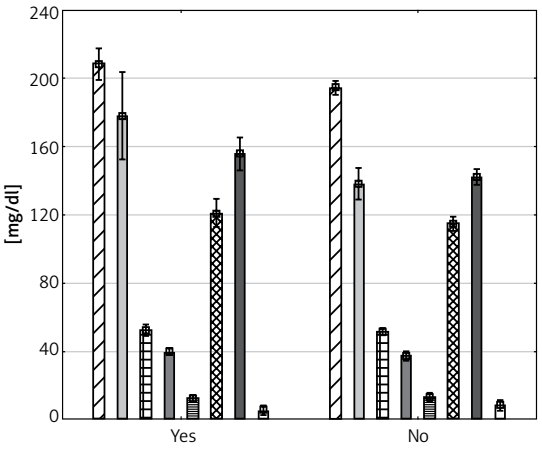
Independent variable	*P*-value	Dependent variables
Place of residence (urban/rural)	< 0.001	CETP β-coefficient (95% CI) 0.18 (0.07 to 0.29)
	< 0.0001	PLTP β-coefficient (95% CI) –0.29 (–0.40 to 0.19)
	0.15	Total C β-coefficient (95% CI) –0.08 (–0.19 to 0.02)
	0.56	LDL-C β-coefficient (95% CI) –0.03 (–0.14 to 0.07)
	0.15	HDL-C β-coefficient (95% CI) –0.08 (–0.19 to 0.02)
	0.16	HDL_2-C β-coefficient (95% CI) –0.07 (–0.18 to 0.03)
	0.22	HDL_3-C β-coefficient (95% CI) –0.06 (–0.17 to 0.04)
	0.34	Non-HDL-C β-coefficient (95% CI) –0.05 (–0.16 to 0.05)
	0.31	TG β-coefficient (95% CI) –0.05 (–0.16 to 0.05)
	0.21	hs-CRP β-coefficient (95% CI) 0.07 (–0.04 to 0.18)

Multiple linear regressions adjusted for age, BMI, smoking habits, alcohol drinking and co-existence of chronic diseases; CI – confidence interval, p-value in bold letter indicates statistical significance.

Significant β coefficients (95% CI) for CETP (p < 0.001) and PLTP (p < 0.0001) were obtained. On the other hand, no significant influence of place of residence on lipid parameters or hs-CRP was found (Table III).

High values of β coefficients estimated for the impact of main confounders (smoking and alcohol drinking) on lipids were the reason for the exclusion of these two factors from confounders, and treating them, together with place of residence, as independent variables. Smoking increased total cholesterol (p < 0.01), TG (p < 0.001), and non-HDL cholesterol (p < 0.001), whereas moderate alcohol drinking increased total (p < 0.05), HDL (p < 0.001), HDL_2 (p < 0.01) and HDL_3 (p < 0.001) cholesterol levels (Figures 3, 4).

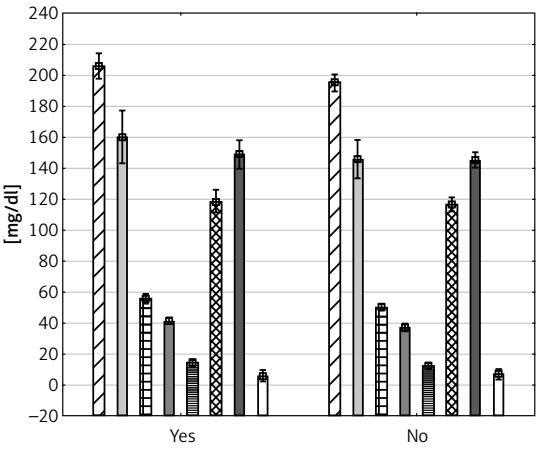
There were no significant differences in CETP or PLTP activity between smokers and non-smokers (CETP: 39.8 ±15.9 vs. 39.3 ±18.7; PLTP: 71.1 ±15.9 vs. 67.1 ±17.3) or alcohol drinkers and non-drinkers (CETP: 41.1 ±16.6 vs. 38.7 ±18.4; PLTP: 68.8 ±18.1 vs. 67.9 ±16.5). Three-way analysis of variance showed that activities of CETP and PLTP, being dependent on place of residence (urban or rural), were not influenced by single confounders, such as smoking or alcohol drinking. However, CETP activity was influenced by the interaction between the two factors smoking and moderate alcohol drinking (Table IV, Figure 5).



CHOL: F (1;421) = 9.6327; p = 0.0020
TG: F (1;421) = 13.245; p = 0.0003
HDL: F (1;421) = 0.4396; p = 0.5077
HDL_3: F (1;420) = 1.5052; p = 0.2206
HDL_2: F (1;420) = 0.1695; p = 0.6807
LDL: F (1;420) = 2.2756; p = 0.1322
Non-HDL: F (1;421) = 8.2194; p = 0.0044
CRP: F (1;421) = 1.6563; p = 0.1988

CHOL TG HDL HDL_3 HDL_2 LDL Non-HDL-C CRP

Figure 3. Lipid pattern and hs-CRP concentration in the group of all men depending on smoking. Charts represent mean ± SE (0.95 CI). Below the box ANOVA test results are presented



CHOL: F (1;421) = 5.5766; p = 0.0187
TG: F (1;421) = 1.7283; p = 0.1893
HDL: F (1;421) = 15.4693; p = 0.00010
HDL_3: F (1;420) = 14.8892; p = 0.0001
HDL_2: F (1;420) = 8.8983; p = 0.0030
LDL: F (1;420) = 0.1345; p = 0.7140
Non-HDL: F (1;421) = 1.1198; p = 0.2906
CRP: F (1;421) = 0.5896; p = 0.4430

CHOL TG HDL HDL_3 HDL_2 LDL Non-HDL-C CRP

Figure 4. Lipid pattern and hs-CRP concentration in the group of all men depending on alcohol consumption. Charts represent mean ± SE (0.95 CI). Below the box ANOVA test results are presented

Table IV. Effect of smoking and/or alcohol drinking and/or site of residence (urban/rural) on CETP and PLTP activity

Effect	*F*-value	*P*-value
Tests of significance for CETP:		
Smoking	0.005	0.946
Drinking	0.710	0.399
Place of residence	10.87	0.001
Smoking*drinking	6.853	0.009
Smoking*residence	0.002	0.965
Drinking*residence	1.927	0.165
Smoking*drinking*residence	0.213	0.644
Tests of significance for PLTP:		
Smoking	0.657	0.418
Drinking	0.041	0.839
Place of residence	30.74	< 0.001
Smoking*drinking	0.127	0.721
Smoking*residence	0.893	0.345
Drinking*residence	0.003	0.955
Smoking*drinking*residence	0.390	0.532

Spreadsheet of three-way analysis of variance. F – F-test value for the respective effects; p – p-value.

In the study, neither coexistence of chronic diseases (such as T2DM or CHD) nor chronic treatment with pharmacological drugs had a significant impact on lipid pattern. Probably, it may be attributed to the relatively small percentage of men diagnosed with these diseases (less than 10%) and the small number of subjects treated with statin (10) or fibrate (only 2).

At the next stage, analysis of CETP and PLTP activity depending on presence of lipid disorders (hypercholesterolemia or atherogenic dyslipidemia) was performed for urban and rural populations of men.

In the group of 151 normolipidemic men, in comparison to urban residents, rural men were characterized not only by lower CETP ($p < 0.001$), higher PLTP ($p < 0.001$) and higher hs-CRP ($p < 0.05$), but also by higher ($p < 0.01$) HDL and HDL_3 cholesterol levels. Multiple regression analysis showed the existence of an inverse correlation between CETP and PLTP ($r = –0.210$; $p < 0.01$).

In the group of 186 hypercholesterolemic subjects, higher CETP activity in comparison to men with normal total cholesterol ($p < 0.001$) was observed. In comparison to urban men, rural men displayed, together with lower CETP ($p < 0.05$) and higher PLTP ($p < 0.001$) activity, a higher total cholesterol level ($p < 0.01$). PLTP activity was positively, significantly correlated with total cholesterol ($r = 0.221$; $p < 0.01$), TG ($r = 0.174$; $p < 0.05$), HDL_2 ($r = 0.153$; $p < 0.05$) and hs-CRP ($r = 0.184$; $p = 0.01$).

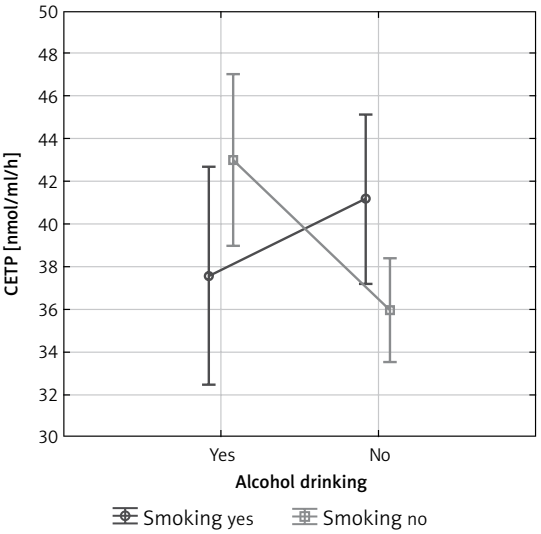


Figure 5. Dependence of CETP activity on interaction between smoking and alcohol drinking ($p < 0.05$)

Also, in the group of 63 men diagnosed with residual dyslipidemia, in comparison to urban residents, the rural men were characterized by lower CETP ($p < 0.05$) and higher PLTP ($p < 0.05$) activity. Other parameters were similar in inhabitants of both urban and rural areas (Table V). Multiple regression analysis showed a statistically significant ($p < 0.05$) linear correlation between CETP and TG ($r = 0.291$), PLTP and HDL-C ($r = 0.271$) and between PLTP and HDL_3-C ($r = 0.287$).

A regression analysis showed no correlation between hs-CRP and CETP or PLTP activity ($r = 0.070$ and $r = 0.057$, respectively).

Lastly, analysis of CETP and PLTP activity in relation to HDL cholesterol level was carried out. The total studied population was divided into three groups, depending on values of HDL-C concentrations: I (higher than/equal to 1.5 mmol/l), II (between 1.0 and 1.5 mmol/l) and III (lower than/equal to 1.0 mmol/l). The total cholesterol level was 5.37 ±1.01 mmol/l in the first group, 5.17 ±1.19 mmol/l in the second group, and 4.86 ±1.03 mmol/l in the third group. The CETP and PLTP activity values in all these groups are presented in Figure 6. The HDL_3-C to HDL_2-C ratio was 3.3 ±6.2 in group I, 3.4 ±1.7 in group II, and 4.7 ±3.1 in group III (the difference in HDL_3-C to HDL_2-C ratio between groups II and III was statistically significant ($p < 0.05$), and the difference between groups I and III was of borderline significance ($p = 0.05$).

Discussion

According to the PURE study, in middle-income and low-income countries, the cardiovascular risk

Table V. Lipid pattern, lipid transfer protein activity and hs-CRP level in urban and rural men depending on lipid pattern

Parameter	Normolipidemia		Hypercholesterolemia		Residual dyslipidemia	
	City (*n* = 104)	Village (*n* = 47)	City (*n* = 112)	Village (*n* = 74)	City (*n* = 34)	Village (*n* = 29)
Age [years]	51.3 ±6.4	50.6 ±5.9	52.0 ±5.8	50.3 ±5.9	53.4 ±5.2	51.2 ±6.2
BMI [kg/m^2]	27.6 ±4.8	28.7 ±5.2	27.9 ±3.5	29.1 ±5.7	30.6 ±3.6	30.2 ±4.5
Smokers, *n* (%)	12 (11.5)	15 (31.9)	32 (28.5)	34 (45.9)	8 (29.6)	11 (39.2)
Drinkers, *n* (%)	26 (25.0)	13 (27.6)	37 (33.0)	28 (37.8)	4 (14.8)	5 (17.8)
DM, *n* (%)	10 (9.6)	1 (2.2)	6 (5.3)	4 (5.4)	1 (3.7)	0 (0)
CHD, *n* (%)	11 (10.5)	2 (4.2)	4 (3.5)	0 (0)	0 (0)	0 (0)
Total C [mmol/l]	4.41 ±0.51	4.43 ±0.53	5.94 ±0.79	6.31 ±1.13**	5.17 ±1.07	4.93 ±0.93
LDL-C [mmol/l]	2.52 ±0.56	2.44 ±0.61	3.70 ±0.74	3.89 ±1.03	2.92 ±1.17	2.71 ±0.95
HDL-C [mmol/l]	1.41 ±0.27	1.57 ±0.48**	1.37 ±0.3	1.45 ±0.44	0.91 ±0.12	0.91 ±0.1
HDL_2-C [mmol/l]	0.35 ±0.14	0.41 ±0.2	0.36 ±0.14	0.38 ±0.19	0.21 ±0.06	0.19 ±0.06
HDL_3-C [mmol/l]	1.06 ±0.2	1.16 ±0.29**	1.01 ±0.21	1.07 ±0.29	0.70 ±0.1	0.72 ±0.08
Non-HDL-C [mmol/l]	3.0 ±0.56	2.85 ±0.61	4.57 ±0.86	4.86 ±1.18	4.24 ±1.06	4.08 ±0.91
TG [mmol/l]	1.08 ±0.31	0.98 ±0.56	1.91 ±1.05	2.15 ±1.70	3.10 ±2.01	2.86 ±1.31
hs-CRP [mg/l]	1.3 ±2.0	3.4 ±10.4*	1.4 ±1.5	1.9 ±2.1	1.9 ±1.8	2.4 ±2.1
CETP [nmol/ml/h]	38.1 ±14.9	28.7 ±15.5***	47.1 ±18.9	40.9 ±17.5*	42.1 ±16.4	32.4 ±18.9*
PLTP [nmol/ml/h]	63.4 ±16.2	75.5 ±17.7***	63.9 ±15.1	75.0 ±18.1***	64.8 ±15.1	72.8 ±15.7*

,,***Statistically significant differences between rural and respective urban male group; *p < 0.05; **p < 0.01; ***p < 0.001.*

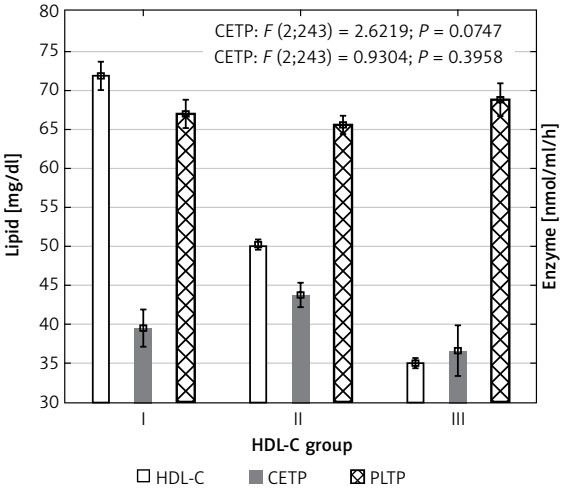


Figure 6. HDL cholesterol level (mg/dl), CETP activity (nmol/ml/h) and PLTP activity (nmol/ml/h) in the groups of men depending on HDL-C value: I – in the range of HDL-C ≥ 60 mg/dl (≥ 1.5 mmol/l; *n* = 103), II – in the range of HDL-C between 60 and 40 mg/dl (1.0–1.5 mmol/l; *n* = 247), III – in the range of HDL-C ≤ 40 mg/dl (≤ 1.0; *n* = 77). Charts represent mean ± SE (0.95 CI). In the box ANOVA test results. Statistical significance between the groups for CETP activity: I vs. III: $p = 0.05$; II vs. III: $p < 0.01$

determined using the INTERHEART risk score is higher in cities, whereas in high-income countries, it is higher in rural areas [23]. Poland belongs to the middle-income countries. The Polish population is characterized by a high proportion of subjects with high (≥ 5%) individual 10-year risk of cardiovascular death (global risk). In 2003–2005 such risk was found in 46% of men and 21% of women. In the Lower Silesia region, the proportion of men with high global risk was 39.9% [20]. The most relevant finding of this study demonstrates differences in CETP and PLTP activity in rural vs. urban living male subjects. With a similar lipid pattern in both groups of men, in men living in villages CETP activity was lower, while PLTP activity was higher than in men living in cities. These differences did not depend on age (which was similar in both groups), BMI, hs-CRP, smoking habits, alcohol drinking, or co-existing chronic diseases such as diabetes mellitus or coronary heart disease. Among confounders, only two were different in groups of urban and rural men: BMI and hs-CRP. Both of these factors were higher in rural men than in urban men.

Elevated BMI in rural men may indirectly indicate some nutritional causes of increase in PLTP activity. Cheung *et al.* found that in women (but not in men) plasma PLTP activity was positively

correlated with lipids and BMI [24]. Murdoch *et al.* reported that elevated PLTP activity in obese subjects can be a result of increased body fat and is influenced by non-esterified fatty acids (NEFAs) [25]. Daily NEFA consumption in rural men was habitually higher than in urban habitants (unpublished data), so it can explain higher PLTP activity in rural men. Nutritional genomics studies also demonstrated interactions between diet and CETP gene polymorphisms [26–28]. It is probable that dietary factors, possibly combined with alcohol consumption and smoking, and interactions that occur between them, were responsible for the increase in PLTP and decrease in CETP activity in the rural population of men as compared to men living in urban areas. Regardless of genetic factors, the causes of observed changes could also be related to inflammatory factors. The serum level of hs-CRP was higher in men from rural than urban areas. However, in the multivariate regression model, hs-CRP was not associated with CETP or PLTP activity. This could be due to the fact that normolipidemic men constituted the highest percentage of respondents. The association between hs-CRP and lipid transfer protein was observed in other studies in groups of people with dyslipidemia. This association was related mainly to PLTP and was expressed as a positive relationship between hs-CRP and PLTP activity [15, 16].

Apart from BMI and hs-CRP, the remaining confounders, especially smoking habits and alcohol drinking, did not influence CETP or PLTP activity. After adjustment for body mass index and hs-CRP, the lower CETP and higher PLTP activity in rural men persisted as statistically significant differences in comparison to urban men ($p < 0.001$ and $p < 0.0001$ for CETP and PLTP, respectively). There was a further question of how these differences may be associated with different lipid patterns in the studied groups. Therefore, an analysis of the activity of CETP and PLTP was performed in groups of men living in an urban or rural area, and diagnosed with normolipidemia or hypercholesterolemia or residual dyslipidemia. In each of these groups, rural men displayed significantly lower CETP and higher PLTP activity compared to urban men.

Of course, relying solely on this study, it is difficult to provide a mechanistic explanation for our findings. Nevertheless, the determination of both CETP and PLTP activity and the relationship between them is the highlight of the study. The relationships between lipids and lipid transfer proteins, such as the significant, positive correlation of CETP with total cholesterol, LDL cholesterol, and non-HDL cholesterol, have also been found in other studies [3, 23]. Similarly to others [29, 30], we found significantly higher CETP activity in hypercholesterolemic vs. normocholesterolemic men. The presence of these relations is consistent with the hypothesis of the pro-atherogenic role of elevated serum CETP activity, as LDL- and non-HDL cholesterol remain the main risk factor for cardiovascular disease [31]. However, we did not find a negative linear correlation between CETP and HDL-C, although increased CETP activity is recognized as a factor associated with decreased HDL cholesterol [6, 12]. The inverse association between CETP activity and HDL-C level appeared in the comparative analysis of lipid transfer protein levels performed depending on the concentration of HDL-C. In the range of normal vs. increased serum HDL-C levels, CETP activity was normal vs. reduced. There was no such dependence between CETP and HDL-C in the range of normal vs. low HDL-C levels; low HDL-C was associated with low CETP activity (Figure 6). PLTP activity was similar in all groups. These disparities showed that CETP-HDL interaction depends on the range of HDL cholesterol levels. It also suggests that the effectiveness of CETP inhibitors in lowering total or LDL cholesterol concentrations may be dependent on the initial HDL cholesterol concentration.

Moreover, it seems that in men with a high HDL cholesterol level not all HDLs were subjected to the CETP action. This can result in a relatively low HDL_3 to HDL_2 cholesterol ratio in men with high HDL levels (similar to the one estimated in the group of men with normal HDL levels). Small HDL_3 particles, containing an increased content of oxidized fatty acids, seem to have impaired anti-inflammatory and antioxidant activities, which were associated with oxidative stress. These dysfunctional HDL particles may be a novel biomarker of cardiovascular risk [32, 33].

A more obvious association than in the case of CETP-HDL was revealed between PLTP activity and HDL cholesterol level, although it appeared only in groups of men with lipid disturbances. This was not surprising, as PLTP, along with CETP, is one of the main factors regulating the size and composition of HDL particles, and controlling plasma HDL levels [34]. In our study, in hypercholesterolemic men, PLTP activity was significantly and positively correlated with total and HDL_2 cholesterol. In men with residual dyslipidemia, a positive correlation between PLTP and HDL-C and between PLTP and HDL_3-C was observed. In other studies human plasma PLTP activity was either positively [22] or negatively [35] correlated with HDL levels. It depended on various exogenous and endogenous factors, including genetic variation of the PLTP gene [26, 35]. This study confirmed the impact of BMI on PLTP activity. A similar observation was described by Murdoch *et al.*, who also found a significant, positive relationship of BMI with PLTP and PLTP with HDL_2 cholesterol. The latter correlation appeared after adjustment of HDL_2 cholesterol for the presence of obesity [36]. In diabetic or obese

patients, plasma leptin levels were related to plasma CETP mass and PLTP activity, which may explain the relationship of BMI with lipid transfer proteins [37, 38]. However, in our study, presence of T2DM was sporadic, and the existence of a PLTP-HDL_2 cholesterol relationship was dependent on cholesterol level rather than obesity. A significant correlation between PLTP (CETP) and TG, observed in hypercholesterolemic and hypertriglyceridemic men (while absent in normolipidemic men), indicates a close association between lipid transfer protein and changed lipid pattern. It is important that as a consequence of elevated PLTP (and elevated CETP) activity, functionally deficient HDL particles, enriched in core TG and depleted in CE and apoA-I, are formed intravascularly [32, 33]. Thus, although therapeutic elevation of plasma HDL has become a major pharmacological target in patients with metabolic diseases, the normalization of HDL functionality should be another aim of therapy [39].

To summarize, our results showed that the existence of a relationship between PLTP and CETP, and between both proteins and lipids, varies along with changes in lipid pattern. The participation of PLTP and CETP in disturbed lipid homeostasis in men with hyperlipidemia resulted in lack of an inverse correlation between CETP and PLTP in men with hypercholesterolemia or atherogenic dyslipidemia. Such a correlation was present in normolipidemic men. Interestingly, also in normolipidemic rats, changes in lipid transfer proteins induced by various polyunsaturated fatty acids confirmed the negative relationship between CETP and PLTP activities [40].

Dullaart *et al.* suggest that higher lipid transfer protein activities may provide a mechanism that contributes to a more atherogenic lipid profile, associated with cigarette smoking [41]. This profile is characterized by higher total cholesterol and triglycerides with lower levels of HDL cholesterol [42, 43]. In the present study, smokers displayed higher total and non-HDL cholesterol, as well as triglycerides, in comparison to non-smokers. However, there was no effect of smoking on HDL or lipid transfer proteins. Other authors have observed decreased CETP or/and PLTP activity in smokers, as compared with non-smokers [44, 45], but their studies were performed in small groups of about a dozen people, whereas our study included 427 men. In our study, also no effect of moderate alcohol consumption on CETP or PLTP activity was found. Likewise, He *et al.* found no significant changes in the activity of lipid transfer proteins in people who drink alcohol moderately [4]. Other authors have reported lower CETP activity among young men drinking alcohol [7, 46, 47]. Genetic variation in the gene encoding CETP may be an important determinant of its activity in men drinking alcohol [48]. In our study, CETP activity showed an interaction effect between smoking and moderate alcohol drinking ($p = 0.009$). This interaction seems to be antagonistic.

To summarize, in the middle-aged male population of the Lower Silesia region in Poland, place of residence (urban or rural area) had a significant impact on the activity of lipid transfer proteins. After adjustment for age, BMI, smoking habits, alcohol drinking, co-existence of lipid disturbances or chronic diseases, and inflammatory state, the lower CETP and higher PLTP activity in rural men persisted as significantly different in comparison to urban men. There were no effects of smoking or moderate alcohol consumption on the lipid transfer proteins; however, CETP activity showed an interaction effect between these two factors. Recently, on the basis of the Framingham Heart Study it was shown that low CETP and high PLTP activities, each independently, predicted cardiovascular events. The combination of both low CETP and high PLTP resulted in higher cardiovascular risk than either of these factors alone. However, both low CETP and high PLTP predicted cardiovascular diseases only in men [21]. According to these results, in the Lower Silesia region, male habitants of villages have greater cardiovascular risk than habitants of cities. Nevertheless, on the basis of a large South Asian population study, it was suggested that elevated CETP activity is a major determinant of atherosclerotic cardiovascular disease [5]. According to these results, urban men could have a greater cardiovascular risk, which was also pointed out in the PURE study [23]. These differences in the interpretation can result from the specifics of the studied populations (race, sex, age, BMI, other confounding factors) and – probably the most important – different lipid patterns. The practical implication of this study, which was performed in groups of men with similar lipid levels, may be the identification of changes in CETP and PLTP activities as one of the mechanisms of high global cardiovascular risk, existing in nearly 40% of the population of men living in the Lower Silesia region.

In conclusion, co-existing differences in CETP and PLTP activities, observed between urban and rural populations of men living in the Lower Silesia region, may be associated with cardiovascular risk. Changes in lipid transfer proteins are probably caused by nutritional and/or genetic factors.

Conflict of interest

The authors declare no conflict of interest.

References

1. van Tol A. Phospholipid transfer protein. Curr Opin Lipidol 2002; 13: 135-9.

2. Quintao EC, Cazita PM. Lipid transfer proteins: past, present and perspectives. Atherosclerosis 2010; 209: 1-9.
3. Tato F, Vega GL, Grundy SM. Bimodal distribution of cholesteryl ester transfer protein activities in normotriglyceridemic men with low HDL cholesterol concentrations. Arterioscler Thromb Vasc Biol 1995; 15: 446-51.
4. He BM, Zhao SP, Peng ZY. Effects of cigarette smoking on HDL quantity and function: implications for atherosclerosis. J Cell Biochem 2013; 114: 2431-6.
5. Rashid S, Sniderman A, Melone M, et al. Elevated cholesteryl ester transfer protein (CETP) activity, a major determinant of the atherogenic dyslipidemia, and atherosclerotic cardiovascular disease in South Asians. Eur J Prev Cardiol 2015; 22: 468-77.
6. Durrington PN. Cholesteryl ester transfer protein (CETP) inhibitors. Br J Cardiol 2012; 19: 126-33.
7. Barter PJ, Brewer HB, Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: novel target for raising HDL and inhibiting atherosclerosis. Arterioscler Thromb Vasc Biol 2003; 23: 160-7.
8. Davidson MH. HDL and CEPT inhibition: will this DEFINE the future? Curr Treat Option Cardiovasc Med 2012; 14: 384-90.
9. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007; 357: 2109-22.
10. Mabuchi H, Nohara A, Inazu A. Cholesteryl ester transfer protein (CETP) deficiency and CETP inhibitors. Mol Cells 2014; 37: 777-84.
11. van Haperen R, van Tol A, van Gent T, et al. Increased risk of atherosclerosis by elevated plasma levels of phospholipid transfer protein. J Biol Chem 2002; 277: 48938-43.
12. Masson D, Jiang XC, Lagrost L, Tall AR. The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res 2009; 50: S201-6.
13. Kärkkäinen M, Oka T, Olkkonen VM, et al. Isolation and partial characterization of the inactive and active forms of human plasma phospholipid transfer protein (PLTP). J Biol Chem 2002; 277: 15413-8.
14. Jiang XC, Jin W, Hussain MM. The impact of phospholipid transfer protein (PLTP) on lipoprotein metabolism. Nutr Metab 2012; 9: 75.
15. Cheung MC, Brown BG, Marino Larsen EK, Frutkin AD, O'Brien KD, Albers JJ. Phospholipid transfer protein activity is associated with inflammatory markers in patients with cardiovascular disease. Biochim Biophys Acta 2006; 1762: 131-7.
16. Tzotzas T, Desrumaux C, Lagrost L. Plasma phospholipid transfer protein (PLTP): review of an emerging cardiometabolic risk factor. Obes Rev 2009; 10: 403-11.
17. Dullaart RP, Vergeer M, de Vries R, Kappelle PJ, Dallinga-Thie GM. Type 2 diabetes mellitus interacts with obesity and common variations in PLTP to affect plasma phospholipid transfer protein activity. J Intern Med 2012; 271: 490-8.
18. Qin S, Kawano K, Bruce C, et al. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. J Lipid Res 2000; 41: 269-76.
19. Skoczyńska A, Wojakowska A, Turczyn B, et al. Lipid pattern in middle-aged inhabitants of the Lower Silesian region of Poland. The PURE Poland sub-study. Ann Agric Environ Med 2013; 20: 317-24.
20. Piwońska A, Piotrowski W, Broda G. Ten-year risk of fatal cardiovascular disease in the Polish population and medical care. Results of the WOBASZ study. Kardiol Pol 2010; 68: 672-7.
21. Robins SJ, Lyass A, Brocia RW, Massaro JM, Vasan RS. Plasma lipid transfer proteins and cardiovascular disease. The Framingham Heart Study. Atherosclerosis 2013; 228: 230-6.
22. Chen X, Sun A, Mansoor A, et al. Plasma PLTP activity is inversely associated with HDL-C levels. Nutr Metab 2009; 6: 49.
23. Yusuf S, Rangarajan S, Teo K, et al. Cardiovascular risk and events in 17 low-, middle-, and high-income countries. N Engl J Med 2014; 371: 818-27.
24. Cheung MC, Knopp RH, Retzlaff B, Kennedy H, Wolfbauer G, Albers JJ. Association of phospholipid transfer protein activity with IDL and buoyant LDL: impact of gender and adiposity. Biochim Biophys Acta 2002; 1587: 53-9.
25. Murdoch SJ, Kahn SE, Albers JJ, Brunzell JD, Purnell JQ. PLTP activity decreases with weight loss: changes in PLTP are associated with changes in subcutaneous fat and FFA but not IAF or insulin sensitivity. J Lipid Res 2003; 44: 1705-12.
26. Perez-Martinez P, Delgado-Lista J, Perez-Jimenez F, Lopez-Miranda J. Update on genetics of postprandial lipemia. Atheroscler Suppl 2010; 11: 39-43.
27. Shin SK, Ha TY, McGregor RA, Choi MS. Long-term curcumin administration protects against atherosclerosis via hepatic regulation of lipoprotein cholesterol metabolism. Mol Nutr Food Res 2011; 55: 1829-40.
28. Ros E, Martínez-González MA, Estruch R, et al. Mediterranean diet and cardiovascular health: teachings of the PREDIMED study. Adv Nutr 2014; 5: 330S-6S.
29. Desrumaux C, Athias A, Besse'de G, et al. Mass concentration of plasma phospholipid transfer protein in normolipidemic, type IIa hyperlipidemic, type IIb hyperlipidemic, and non-insulin-dependent diabetic subjects as measured by a specific ELISA. Arterioscler Thromb Vasc Biol 1999; 19: 266-75.
30. Sasai K, Okumura-Noji K, Hibino T, et al. Human cholesteryl ester transfer protein measured by enzyme-linked immunosorbent assay with two monoclonal antibodies against rabbit cholesteryl ester transfer protein: plasma cholesteryl ester transfer protein and lipoproteins among Japanese hypercholesterolemic patients. Clin Chem 1998; 44: 1466-73.
31. Adhyaru BB, Jacobson TA. Atherosclerotic cardiovascular disease risk: a comparison of the 2013 American College of Cardiology/American Heart Association Cholesterol Guidelines with the 2014 National Lipid Association Recommendations for Patient-Centered Management of Dyslipidemia. Cardiol Clin 2015; 33: 181-96.
32. Otocka-Kmiecik A, Mikhailidis DP, Nicholls SJ, Davidson M, Rysz J, Banach M. Dysfunctional HDL: a novel important diagnostic and therapeutic target in cardiovascular disease? Prog Lipid Res 2012; 51: 314-24.
33. Toth PP, Barylski M, Nikolic D, Rizzo M, Montalto G, Banach M. Should low high-density lipoprotein cholesterol (HDL-C) be treated? Best Pract Res Clin Endocrinol Metab 2014; 28: 353-68.
34. Huuskonen J, Olkkonen VM, Jauhiainen M, Ehnholm C. The impact of phospholipid transfer protein (PLTP) on HDL metabolism. Atherosclerosis 2001; 155: 269-81.
35. Tahvanainen E, Jauhiainen M, Funke H, Vartiainen E, Sundvall J, Ehnholm C. Serum phospholipid transfer protein activity and genetic variation of the PLTP gene. Atherosclerosis 1999; 146: 107-15.
36. Murdoch SJ, Carr MC, Hokanson JE, Brunzell JD, Albers JJ. PLTP activity in premenopausal women. Rela-

tionship with lipoprotein lipase, HDL, LDL, body fat, and insulin resistance. J Lipid Res 2000; 41: 237-44.
37. Dullaart RP, de Vries R, Dallinga-Thie GM, van Tol A, Sluiter WJ. Plasma cholesteryl ester transfer protein mass and phospholipid transfer protein activity are associated with leptin in type-2 diabetes mellitus. Biochim Biophys Acta 2007; 1771: 113-8.
38. Tzotzas T, Dumont L, Triantos A, Karamouzis M, Constantinidis T, Lagrost L. Early decreases in plasma lipid transfer proteins during weight reduction. Obesity 2006; 14: 1038-45.
39. Chapman MJ, Le Goff W, Guerin M, Kontush A. Cholesteryl ester transfer protein: at the heart of the action of lipid-modulating therapy with statins, fibrates, niacin, and cholesteryl ester transfer protein inhibitors. Eur Heart J 2010; 31: 149-64.
40. Skoczyńska A, Wojakowska A, Nowacki D, et al. Unsaturated fatty acids supplementation reduces blood lead level in rats. Biomed Res Int 2015; 2015: 189190.
41. Dullaart RP, Hoogenberg K, Dikkeschei BD, van Tol A. Higher plasma lipid transfer protein activities and unfavorable lipoprotein changes in cigarette-smoking men. Arterioscler Thromb 1994; 14: 1581-5.
42. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. BMJ 1989; 298: 784-8.
43. Gossett LK, Johnson HM, Piper ME, et al. Smoking intensity and lipoprotein abnormalities in active smokers. J Clin Lipidol 2009; 3: 372-8.
44. Mero N, van Tol, Scheek LM, et al. Decreased postprandial high density lipoprotein cholesterol and apolipoproteins A-I and E in normolipidemic smoking men: relations with lipid transfer proteins and LCAT activities. J Lipid Res 1998; 39: 1493-502.
45. Zaratin AC, Quintão EC, Sposito AC, et al. Smoking prevents the intravascular remodeling of high-density lipoprotein particles: implications for reverse cholesterol transport. Metabolism 2004; 53: 858-62.
46. Hannuksela M, Marcel YL, Kesaniemi YA, Savolainen MJ. Reduction in the concentration and activity of plasma cholesteryl ester transfer protein by alcohol. J Lipid Res 1992; 33: 737-44.
47. Boekholdt SM, Sacks FM, Jukema JW, et al. Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. Circulation 2005; 111: 278-87.
48. Jensen MK, Mukamal KJ, Overvad K, Rimm EB. Alcohol consumption, TaqIB polymorphism of cholesteryl ester transfer protein, high-density lipoprotein cholesterol, and risk of coronary heart disease in men and women. Eur Heart J 2008; 29: 104-12.