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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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-
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 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]. Dalce-trapib 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/m²). 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

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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 and HDL-C < 1.0 mmol/l and a normal lipid pattern (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/S (Friedewald formula). The QUANTOLIP HDL (Technoclone GmbH, Vienna, Austria) precipitation test was used to measure HDLₐ and HDLₐ 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 HITACHIF-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/m² was treated as normal, > 25 kg/m² as overweight, > 30 kg/m² 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).

### 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/m²]      | 27.7 (25.4–30.5)       | 28.4 (26.1–31.5)*     |
| WHR, mean ± SD [kg/m²]         | 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$_{3}$-C, mean ± SD [mmol/l]| 0.34 ± 0.14            | 0.35 ± 0.19            |
| HDL$_{2}$-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.

### 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; $p < 0.001$. 

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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), HDL₂ (p < 0.01) and HDL₃ (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).

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 | HDL₂-C β-coefficient (95% CI) −0.07 (−0.18 to 0.03) |
|                       | 0.22 | HDL₃-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 (0.01 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.
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 \(\left( r = -0.210; \ p < 0.01 \right)\).

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 \(\left( r = 0.221; \ p < 0.01 \right),\) TG \(\left( r = 0.174; \ p < 0.05 \right),\) HDL\(_2\) \(\left( r = 0.153; \ p < 0.05 \right)\) and hs-CRP \(\left( r = 0.184; \ p = 0.01 \right)\).

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 \(\left( r = 0.291 \right)\), PLTP and HDL-C \(\left( r = 0.271 \right)\) and between PLTP and HDL\(_3\)-C \(\left( r = 0.287 \right)\).

A regression analysis showed no correlation between hs-CRP and CETP or PLTP activity \(\left( r = 0.070 \right)\) and \(\left( r = 0.057 \right)\), 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 \(5.37 \pm 1.01\) mmol/l in the first group, \(5.17 \pm 1.19\) mmol/l in the second group, and \(4.86 \pm 1.03\) mmol/l in the third group. The CETP and PLTP activity values in all these groups are presented in Figure 6. The HDL\(_1\)-C to HDL\(_2\)-C ratio was \(3.3 \pm 6.2\) in group I, \(3.4 \pm 1.7\) in group II, and \(4.7 \pm 3.1\) in group III (the difference in HDL\(_1\)-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...
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

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)     |
| Age (years)                | 51.3 ±6.4               | 50.6 ±5.9            | 52.0 ±5.8             | 50.3 ±5.9            |
| BMI [kg/m²]                | 27.6 ±4.8               | 28.7 ±5.2            | 27.9 ±3.5             | 29.1 ±5.7            |
| Smokers, n (%)             | 12 (11.5)               | 15 (31.9)            | 32 (28.5)             | 34 (45.9)            |
| Drinkers, n (%)            | 26 (25.0)               | 13 (27.6)            | 37 (33.0)             | 28 (37.8)            |
| DM, n (%)                  | 10 (9.6)                | 1 (2.2)              | 6 (5.3)               | 4 (5.4)              |
| CHD, n (%)                 | 11 (10.5)               | 2 (4.2)              | 4 (3.5)               | 0 (0)                |
| Total C [mmol/l]           | 4.41 ±0.51              | 4.43 ±0.53           | 5.94 ±0.79            | 6.31 ±1.13**         |
| LDL-C [mmol/l]             | 2.52 ±0.56              | 2.44 ±0.61           | 3.70 ±0.74            | 3.89 ±1.03           |
| HDL-C [mmol/l]             | 1.41 ±0.27              | 1.57 ±0.48**         | 1.37 ±0.3             | 1.45 ±0.44           |
| LDL-C [mmol/l]             | 0.35 ±0.14              | 0.41 ±0.2            | 0.36 ±0.14            | 0.38 ±0.19           |
| HDL-C [mmol/l]             | 1.06 ±0.2               | 1.16 ±0.29**         | 1.01 ±0.21            | 1.07 ±0.29           |
| Non-HDL-C [mmol/l]         | 3.0 ±0.56               | 2.85 ±0.61           | 4.57 ±0.86            | 4.86 ±1.18           |
| TG [mmol/l]                | 1.08 ±0.31              | 0.98 ±0.56           | 1.91 ±1.05            | 2.15 ±1.70           |
| hs-CRP [mg/l]              | 1.3 ±2.0                | 3.4 ±10.4*           | 1.4 ±1.5              | 1.9 ±2.1             |
| CETP [nmol/ml/h]           | 38.1 ±14.9              | 28.7 ±15.5***        | 47.1 ±18.9            | 40.9 ±17.5*          |
| PLTP [nmol/ml/h]           | 63.4 ±16.2              | 75.5 ±17.7***        | 63.9 ±15.1            | 75.0 ±18.1***        |

*,**,**,**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.
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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. 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 to HDL cholesterol ratio in men with high HDL levels (similar to the one estimated in the group of men with normal HDL levels). Small HDL 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 cholesterol. In men with residual dyslipidemia, a positive correlation between PLTP and HDL-C and between PLTP and HDL-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 cholesterol. The latter correlation appeared after adjustment of HDL cholesterol for the presence of obesity [36]. In diabetic or obese
CETP and PLTP activities 

confirmed the negative relationship between induced by various polyunsaturated fatty acids lipidemic rats, changes in lipid transfer proteins normolipidemic men. Interestingly, also in normo-
in men with hypercholesterolemia or atherogenic stasis in men with hyperlipidemia resulted in lack participation of PLTP and CETP in disturbed lipid homeo-
persistance of a relationship between PLTP and CETP , and between both proteins and lipids, varies
pendent on cholesterol level rather than obesity. A significant correlation between PLTP (CETP) and TG, observed in hypercholesterolemic and hyper-
triglyceridemic 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 [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.

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